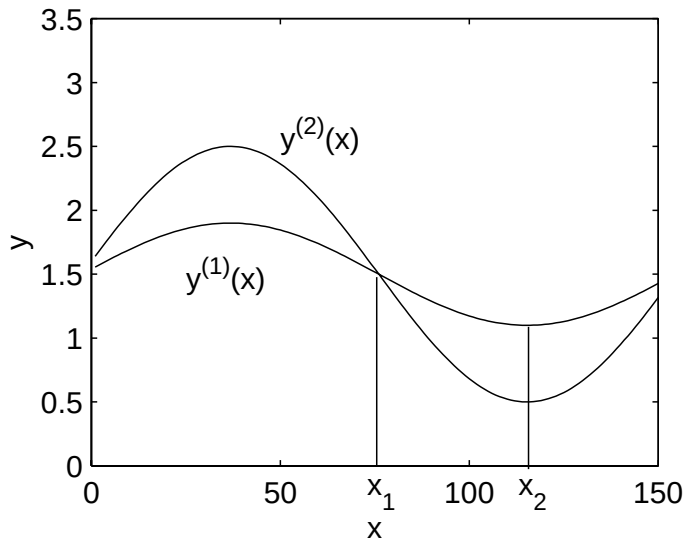

FIG. 3.5. Hunter-Reiner approach for an optimal experiment design to discriminate between two rival models.

3.4 Optimal Experiment Design for Structure Characterisation

3.4.1 *Theoretical Background of OED/SC*

To start with, the OED problem must be clearly stated and translated into an objective function. The goal is, for instance,

To design an experiment to discriminate between rival models y_1 and y_2.

A very useful and intuitive translation into an objective function is given by the Hunter-Reiner approach [130] that chooses the experimental conditions Ψ_i such that the difference between the predictions by both models

$$\left(\hat{y}_1(\Psi_i) - \hat{y}_2(\Psi_i)\right)^2 \tag{3.29}$$

is maximised.

The approach is illustrated in the examples of Figure 3.5. In both cases it is obvious that no selection can be made between the two models in competition at condition 1 while maximal discriminative power is obtained under experimental condition 2.

While this approach is very appealing (and widely applied), a major drawback is that no consideration is given to the fact that uncertainty exists on the model predictions $\hat{y}_i$. Figure 3.6 illustrates the problem of uncertainty effects for two simple regression models that are in competition:

$$y_1 = bx + a \tag{3.30}$$

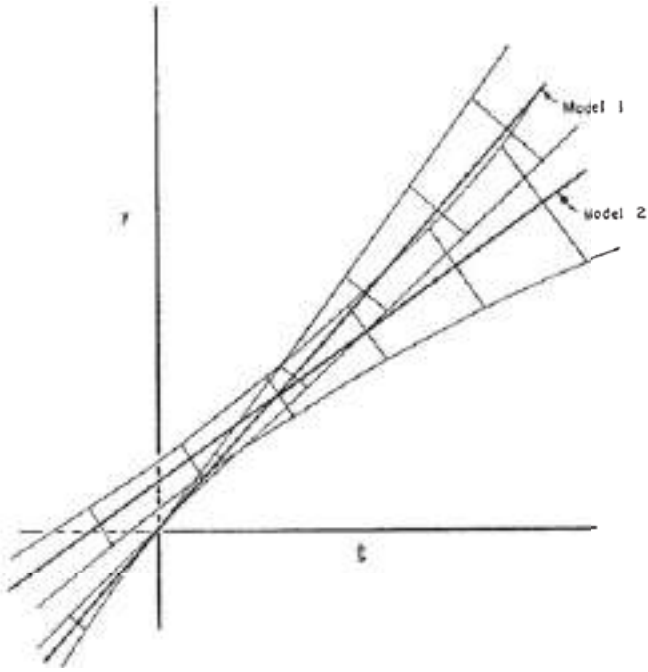

FIG. 3.6. Uncertainty in model predictions of two rival regression models.

$$y_2 = bx \tag{3.31}$$

It is straightforward to compute the confidence regions for both models. From the figure it is evident that the confidence regions are important for the assessment of the discriminative power of an experiment. For this particular case, it is clear that model selection will be most reliable under experimental conditions Ψ when equal or lower than zero. Note that one would have preferred high positive values of the experimental conditions Ψ when the confidence regions were not considered.

Box and Hill [39] developed a quantitative description that allows the computation of the divergence between rival models under model prediction uncertainty. They extended the method to the case of discrimination among m rival models and the use of the combined data of n previous experiments so as to design the $(n+1)$th. It would lead us too far to include these complex objective functions for OED/SC here. They can be found in the mentioned reference together with some illustrative examples.

The OED/SC methods mentioned so far were mainly developed for static models (e.g. regression type empirical models). For dynamic models Munack [181] proposed two approaches, one based on the maximisation of the difference in model predictions, i.e. similar to the Hunter-Reiner method, the other being based

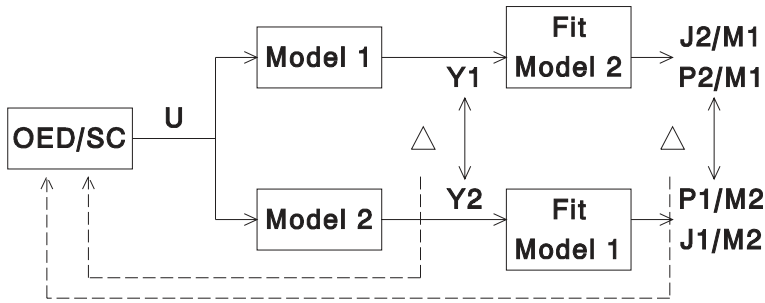

FIG. 3.7. OED/SC: Munack's first approach [181].

on the maximization of the change in parameter estimates when a model is fitted to data obtained under quite different experimental conditions. The latter in fact corresponds to a kind of adequacy evaluation of the rival models since adequate models are characterised by the fact that the parameters do not change with the experimental conditions.

For the first approach the optimisation loop of OED/SC is the following [181] (see Figure 3.7):

1. propose experimental conditions and perform hypothetical experiments through simulation with the 2 models, giving rise to two sets of "raw" data;
2. (a) consider that Model 1 is correct, then fit Model 2 to the Model 1 generated data (evidently, Model 1 must not be fitted since the data originate from this model);
   (b) consider that Model 2 is correct, then fit Model 1 to the data generated from Model 2 (here Model 2 must not be fitted);
3. for both cases calculate the difference between the trajectories simulated with the "best" parameter estimates;
4. maximise the smallest of the two calculated differences (Model 1 - Model 2 fitted), (Model 2 - Model 1 fitted).

It is important to note that the two parameter estimations are included in the procedure, because it is reasonable to expect that a dataset generated by a particular model can be described best by the rival model after a new estimation of its parameters. However, these parameter estimations mean that this OED/SC approach may ask for lengthy computations. These may not be desired for certain applications.

In the second method proposed by Munack [181], the change in the model parameter values needed to fit the data generated in an experiment with different conditions is assessed and maximised by the experiment design. Since no a priori knowledge on the "best" model is available at the moment the experiment design is performed, the rival models have to be treated on an equal basis. The design calcu-

lations are identical to the abovementioned procedure but as an objective function to be maximised the parameter sets for the first (real) experiment and the second (simulated) experiment are compared for both models. For this, the Mahalanobis distance between the parameter vectors is calculated and the smallest distance is maximised. The Mahalanobis distance is a measure of the difference between two vectors which takes the estimation accuracy into account.

3.4.2 *Application: Real-time OED/SC in a Respirometer*

In the respirometer case study developed throughout this chapter, the possibility exists to adjust the experimental conditions applied to the bioreactor. The aim is to maintain the quality of the identification of models describing the biodegradation processes. In this section attention will be given to a specific method that enables OED/SC even under the real-time constraints imposed by the fact that a new optimal experiment for structure characterisation must be designed within the 30 minute interval between consecutive experiments. It was shown that the methods described above could not handle this constraint due to the excessive computational burden and, therefore, a dedicated method had to be developed [261]. It is important to note that, as for every OED/SC method, the objective function is closely related to the method of structure characterisation applied during the identification stage. In the case study, the a priori structure characterisation method based on the number of inflection points is used.

It is good to recall that to determine the number of inflection points from the respirograms and their reliability, the second derivative (the curvature) must be calculated. To estimate its value and to determine whether it is significant, a moving window regression with window width n is applied and both a straight line and a parabola are fitted from points j up to $j + n$.

Whether the parabolic fit is significantly better, and therefore, whether the estimated value of the curvature is significantly different from zero, is tested by [13]:

$$\frac{(SSR_1 - SSR_2)/1}{SSR_2/(n-2)} \simeq F(1; n-2) \tag{3.32}$$

where SSR_1 and SSR_2 are the residuals sum of squares of the linear and parabolic regression respectively. $SSR_1 - SSR_2$ is the extra sum of squares due to the inclusion of the curvature in the regression. The null hypothesis that the parabolic fit is not significantly better than the linear one can be tested by referring this ratio of mean squares to the F-distribution with 1 and $n - 2$ degrees of freedom. The 1 in the numerator is the difference in degrees of freedom between a straight line and a parabola. One should note that, basically, the approach used to discriminate between the parabola and the linear regression is based on the F-test that was presented as one of the traditional structure characterisation methods.

If the parabolic fit is significantly better, the highest order coefficient (the curvature) is returned, otherwise its value is set to zero. An inflection point is defined

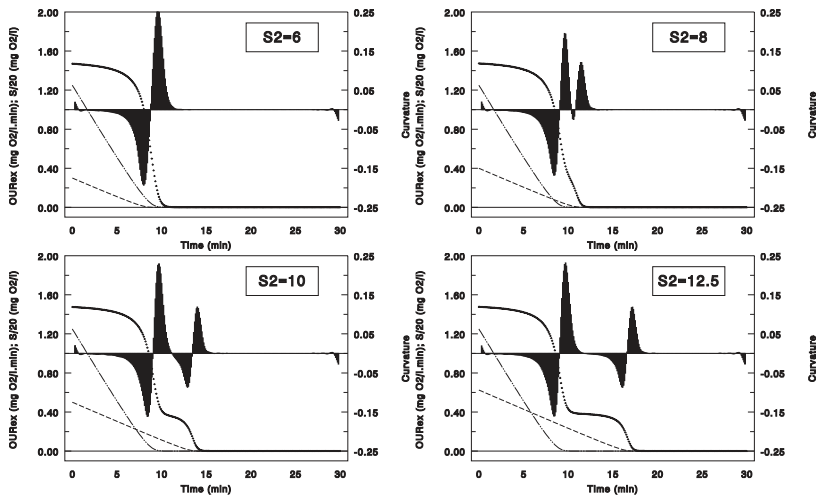

FIG. 3.8. Curvature (filled curve) of respirograms (top dashed lines starting horizontally) obtained with different ratios of two substrates (two dashed lines starting linearly decreasing): $S_1(0) = 25$, $S_2(0) = 6, 8, 10, 12.5$.

by a point where the curvature crosses zero. The results for simulated (noise free) example respirograms are displayed in Figure 3.8.

To define the reliability of an inflection point, it is to be noticed that, as illustrated in Figure 3.8, an inflection point is surrounded with two *pulses*, a positive one and a negative one. These two pulses can be used in several ways to define the reliability $r(f)$ of an inflection point f, e.g.:

- The surface of both pulses
- The total height of both pulses
- The height of the smallest pulse

The first approach will be used in the sequel. In order to increase the discriminative power of the experiments, the aim is to determine the inflection points with the highest reliability, and hence the aim of the optimal experiment design is to maximise $r(f)$.

The examples given will be restricted to the cases where the wastewater influent contains two substrates S_1 and S_2 (hence, a Double Monod model can be used) and the aim is to design an experiment such that the Double Monod model will be reliably selected. Pulse injection of such wastewater to the batch reactor of the respirometer results in initial substrate concentrations noted as $S_1(0)$ and $S_2(0)$. Two related problems will be treated:

OED/SC for Calibrations: In this type of experiment, the experimenter can add a chosen mixture of the two substrates. Since both the initial concentrations

Table 3.2 Two parameter sets used in the simulations for OED/SC

Parameter	Set 1	Set 2
X	4000 mg/l	4000 mg/l
μ_{max1}	5. e-4 /min	2.62 e-4 /min
K_{s1}	1.mg/l	0.226 mg/l
μ_{max2}	1. e-4 /min	2.85 e-4 /min
K_{s2}	0.2 mg/l	0.6 mg/l

$S_1(0)$ and $S_2(0)$ can be chosen by the experimenter, two degrees of freedom for the optimisation problem exist.

OED/SC for Wastewater: In this case, since the wastewater composition can not be altered, the ratio $\frac{S_1(0)}{S_2(0)}$ is fixed. Only the amount of wastewater injected is variable and, hence, only one degree of freedom is left.

OED/SC for Calibrations. In the respirometer case study, calibrations are regularly performed, mainly to verify the correct operation of the measuring device. As shown by Vanrolleghem and Verstraete [266], this calibration can, however, also be used to independently characterise the two main groups of aerobic organisms in activated sludge, i.e. heterotrophs and nitrifiers. To this end, a calibration mixture of ammonia and a readily biodegradable carbon source such as acetate is injected. The optimal experiment design is then aimed at finding the amount of each substrate such that the resulting OUR_{ex}-curve allows the extraction of the three inflection points with the highest reliability and within a short experimentation time. This can be done by maximising the reliability of the three inflection points, or by maximising the least reliable inflection point. The first approach has been chosen and hence the following optimisation problem can be formulated:

$$\max_{S_1(0),S_2(0)} r(f_1) + r(f_2) + r(f_3) \tag{3.33}$$

where $S_1(0)$ and $S_2(0)$ are the initial concentrations of the calibration substrates and $r(f_i)$ is the area of the positive and negative pulses that determine the ith inflection point (see above).

This optimisation problem can be approximately solved by computing the sum of the reliabilities for each substrate combination on the grid $S_1(0)$ = 5(5)50 (from 5 to 50 in steps of 5) and $S_2(0)$ = 5(5)50 mg/l. One obtains a response surface that points to the optimal substrate combination. In Table 3.2, the two sets of biokinetic parameters that were used in the simulations are summarised. For Set 1, the results are schematised in Figure 3.9. On the left, the sum of surfaces is given as a contour-plot. Black indicates the experimental conditions to avoid and the lighter areas result in more reliable inflection points. The length of the respirogram is depicted on the right side. An experimental condition resulting in a respirogram that takes longer than 30 mins to return to an OUR_{ex} = 0 is colored black, reflecting

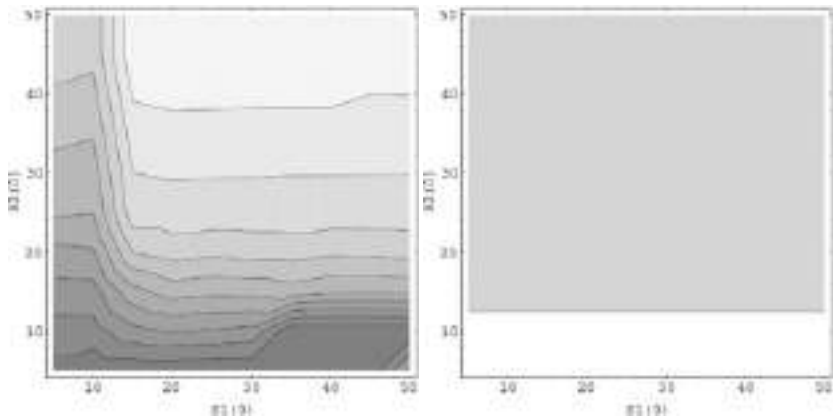

FIG. 3.9. Contourplot indicating the reliability (left) and the length (right) of the respirogram for parameter Set 1. Black indicates lower reliability or a violation of the real time constraint.

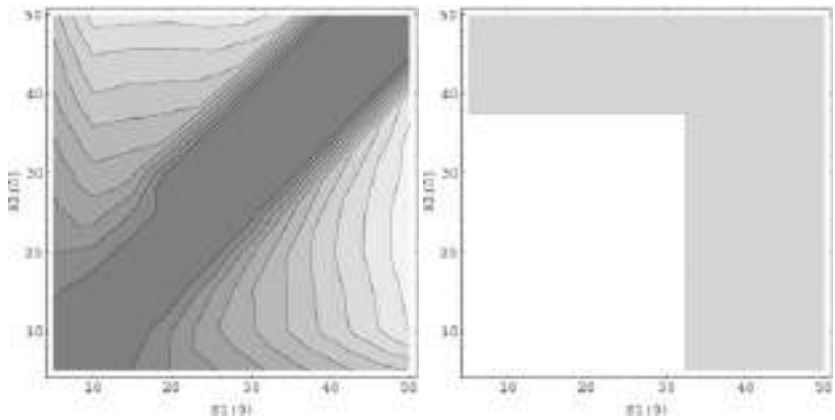

FIG. 3.10. Contourplot indicating the reliability (left) and the length (right) of the respirogram for parameter Set 2. Black indicates lower reliability or a violation of the real time constraint.

the importance given to the real-time constraint in this optimisation problem. From this it is clear that the line $S_2(0) = 5$ mg/l should be avoided and that $S_2(0)$ should be less than 12.5 mg/l.

In Figure 3.8 the OUR_{ex}-curve and corresponding substrate removal curves for four cases with increasing $S_2(0)$ are displayed ($S_1(0)$ is fixed at 25 mg COD/l). Note that the OUR_{ex}-profile in the top left case degenerates in a respirogram typical for a Single Monod model, implying that the parameters of the Double Monod

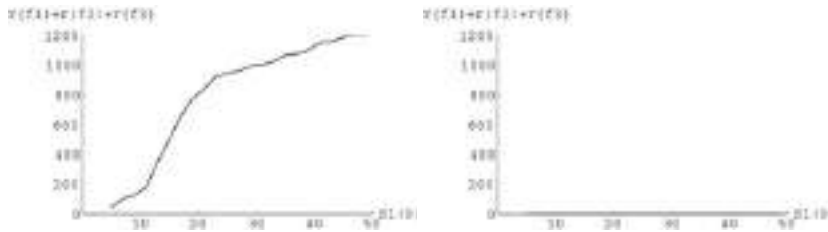

FIG. 3.11. The reliability of inflection point determination for a given $S_1(0)$ and a ratio $\frac{S_1(0)}{S_2(0)} = 1$ for parameter Set 1 (left) and Set 2 (right).

model have become practically unidentifiable from such experimental data [266].

It is very important to realise that this optimal experiment design for SC needs to be performed regularly, because changed biokinetic parameters (e.g. because of biomass adaptation) may result in a completely different advice. This is clearly illustrated by the OED/SC results (Figure 3.10) for the second set of parameter values of Table 3.2. For these sludge characteristics, the substrate concentrations to avoid, i.e. the line $S_1(0) = S_2(0)$, are clearly different compared to the ones obtained for the first parameter set (Figure 3.9).

This result emphasises that the need may exist for certain applications to perform OED/SC on-line. It also stresses the requirement for a priori SC methods so as to meet real-time constraints for timely OED.

OED/SC for Wastewater. The OED/SC for wastewater can be derived from the OED/SC for Calibrations. Suppose the wastewater composition is characterised by a ratio $r = \frac{S_1(0)}{S_2(0)} = \alpha$. This implies that only initial concentrations lying on the line $S_1(0) = \alpha S_2(0)$ need to be considered. This line can be drawn on the given contour plots (Figure 3.9 and Figure 3.10), reducing the two-dimensional contourplot to a one-dimensional plot. One of these is shown in Figure 3.11(left) for the first set of parameters and for a fixed ratio $\frac{S_1(0)}{S_2(0)}$ =1. The reliability of the inflection point $r(f)$ is plotted versus $S_1(0)$, while $S_2(0)$ can be computed from the known ratio. Figure 3.11(left) illustrates that the reliability increases monotonically with increasing $S_1(0)$. The increase, however, is variable, implying that the gain in reliability is not constant. No maximum is found, but $S_1(0)$ is limited by the same real-time constraints as in Figure 3.9, limiting $S_1(0)$ to a maximum of about 12.5 mg/l.

The same wastewater composition (i.e. $S_1(0) = S_2(0)$) with an activated sludge characterised by parameter Set 2, results in respirograms which do not allow selection of the correct (Double Monod) model as illustrated in Figure 3.11(right): no $S_1(0)$ can be found for which the resulting respirogram will yield significant inflection points and, hence, no Double Monod model will be selected from data collected from such an experiment, although the process may intrinsically be a Double Monod type process. Only increasing the degrees of freedom of the exper-

iment design may help to solve the identification problem. This may, for instance, be achieved by an artificial change of the wastewater's composition by deliberate addition of one of the substrate's concentrations (e.g. ammonia).

3.5 Conclusions

In this chapter we have introduced the basic concepts for Structure Characterisation (SC) and illustrated them via a set of different model structure candidates used in respirometry.

We have first made a distinction between a priori and a posteriori structure characterisation methods. Most of the proposed methods use different statistical tests. We have then proposed an approach for the optimal design of experiments in order to obtain the most reliable structure characterisation possible in the context of WWTP models.

The optimal experiment design for structure characterisation will be further developed in Section 5.5 where it will be combined with parameter estimation (OED for SC/PE).

Finally, as an extension of the methods introduced in Chapter 7, we shall introduce another approach for model selection based on the Key Transformation introduced in Chapter 2 (Section 2.8) that allows the selection of a reaction scheme independently of knowledge of the kinetics.

4

Structural Identifiability

4.1 Introduction

The identification of the dynamical models describing wastewater processes is characterised by two important features:

1. The models are most often highly complex, they are usually high-order non linear systems incorporating a large number of state variables and parameters. For instance, the IWA Activated Sludge Model No. 1 ([120], see also Chapter 2) contains 13 state variables and 19 parameters.
2. There is, generally speaking, a lack of cheap and reliable sensors and techniques for measurement of the key state variables. Despite considerable efforts, measurement methodology is still considered to be the weakest part in process modelling and control [111], [265].

Both problems are common to all biotechnological processes, although particularly crucial in wastewater treatment processes, because of the inherent particularly complex nature of these processes, involving for instance many different microbial populations which are often difficult to reliably identify with the available instrumentation.

The values of the parameters in a model are to be inferred from a priori knowledge and experimental data. The quality of the estimation of parameters will depend on the amount and quality of (real-time) data that is available to the identification algorithm. Besides these limitations on the available information, another

problem in the identification of the process model appears: the model parameters may exhibit considerable correlation.

Because of the model complexity and the scarcity of (on-line) sensors, the identifiability study of the dynamical models, prior to any identification, is certainly a key question. The central question of the identifiability analysis is the following:

Assume that a certain number of the state variables are available for measurement; on the basis of the model structure (structural identifiability) or on the type and quality of available data (practical identifiability), can we expect to give via parameter estimation a unique value to the model parameters?

Simply speaking, one would wonder what is the use of trying to calibrate the parameters of a model which is, structurally or practically, unidentifiable. The above formulation is quite crude, but the answer to the identifiability analysis is often more subtle: it is not just a "yes or no" answer, but when it results in some conclusions (what is not a priori obvious with nonlinear models), these may indicate that some subset or combinations of the model parameters are a priori identifiable.

The goal of Chapters 4 and 5 is to study both the structural and practical identifiability of models used in biological wastewater treatment processes. Our intention is to give an introduction to identifiability in the context of wastewater treatment processes. Because the book is basically dedicated to WWTP engineers and not to mathematicians, we have decided to concentrate on the basic concepts without giving a fully rigourous and involved mathematical description. The interested reader can anyway be referred to a number of very good books and papers, e.g. [100], [206], [279], [254], [99]. For illustrative purposes, we shall use different examples including models employing Monod type limitation kinetics. Yet our objective is to deal with the identifiability in a sufficiently general way so as to allow the extension of the proposed study to other practical situations.

The chapter is organised as follows. The theoretical framework of the identification study will be briefly addressed in Section 4.2. It will be further developed in Section 4.3, where some important definitions are reviewed and basic concepts for the structural identifiability tests are introduced. The structural identifiability of the models is studied in Sections 4.4 and 4.5. Six different techniques (Laplace transform, Taylor series expansion, generating series, local state isomorphism, transformation of the nonlinear models, and Lyapunov based analysis) have been considered. The first five are introduced in Sections 4.3 and 4.4 and illustrated on the basis of a simple nonlinear model (yet linear in the parameters). The sixth method (Lyapunov based analysis) is introduced in Section 4.5 in a historical perspective by considering the structural identifiability of the Monod model. Section 4.6 illustrates the structural identifiability concept and tests with respirometer-based models. Finally, Section 4.7 introduces the notion of overparametrisation illustrated with an anaerobic digestion model.

4.2 Theoretical Framework

The notion of structural identifiability is related to the possibility to give a unique value to each parameter of a mathematical model. In simple words, the question of structural identifiability of a model can be formulated as follows (a rigourous definition can be found e.g. in [100]): given a model structure and perfect (i.e. that corresponds perfectly to the model) data of model variables, are all the parameters of the model identifiable? From the structural identifiability analysis one may conclude that only combinations of the model parameters are identifiable. If the number of resulting combinations is lower than the original model parameters, or if there is not a one-to-one relationship between both parameter sets, then a priori knowledge about some parameters may be required to achieve identifiability of each individual parameter. A simple example may illustrate this: in the model $y = ax_1 + bx_2 + c(x_1 + x_2)$, only the parameter combinations $a + c$ and $b + c$ are structurally identifiable (and not the three parameters a, b, c); two parameters (e.g. a and b) will be identifiable if the value of a third one (here, c) is known a priori.

For linear systems, the structural identifiability is rather well understood, and besides classical identifiable models (like dynamical models in canonical form [234], [159], [90]), there exists a number of tests for the identifiability (e.g. Laplace transform method, Taylor series expansion of the observations, Markov parameter matrix approach, modal matrix approach,..., see e.g. [100]). However, for models that are nonlinear in the parameters (like the models studied in this book), the problem is much more complex. Several structural identifiability tests also exist, but they are usually very complex (they typically require the (very helpful) use of symbolic software packages [206], as will be illustrated below).

Practical identifiability on the other hand is related to the quality of the data and their "information" content: are the available data informative enough for identifying the model parameters and for giving accurate values? In the model $y = ax_1 + bx_2$ the parameters are structurally identifiable but they will not be practically identifiable if the experimental conditions are such that the independent variables x_1 and x_2 are always proportional ($x_1 = \alpha x_2$) (then only the combination $a\alpha + b$ is identifiable). This topic (practical identifiability) will be the object of Chapter 5.

4.3 Notion of Structural Identifiability of Linear Systems

4.3.1 *A Simple Example*

Let us start with a simple example. Let us consider a CSTR with one reaction:

$$A \longrightarrow B \tag{4.1}$$

described by first order kinetics. The dynamical mass balance of the substrate A is given by the following equation:

$$\frac{dC}{dt} = -DC + DC_{in} - k_0 C \tag{4.2}$$

with C the concentration of reactant A, C_{in} its influent concentration, D the dilution rate, and k_0 the kinetic constant.

Assume that C and C_{in} are accessible for measurement and that k_0 and D are unknown and constant, i.e. these will be parameters whose value has to be determined.

These two parameters are structurally identifiable. Indeed, if for instance you apply a step of C_{in} to the reactor (Figure 4.1(bottom)), then the step response (Figure 4.1(top)) is equal to:

$$C(t) = C_0 + \frac{D}{D + k_0} \Delta C_{in} (1 - e^{-(D+k_0)t}) \tag{4.3}$$

with C_0 the initial value (before the step) of the reactant concentration C and ΔC_{in} the amplitude of the influent concentration step. From Figure 4.1(top), we note that the amplitude A of the step response of $C(t)$ is equal to the difference between the initial value and the final response value (i.e. after a sufficiently long time). Looking at equation (4.3), we see that A is equal to $\frac{D}{D+k_0}\Delta C_{in}$ (= $C(t = \infty)$ - C_0). Figure 4.1(top) can also be used to compute the time constants corresponding to the dynamics of $C(t)$. Indeed, from equation (4.3), we can deduce that 95 % of the final value of $C(t)$ is reached at a time $t_r = \frac{3}{D+k_0}$ after the time step has been applied. This corresponds to three times the time constant $\tau = \frac{1}{D+k_0}$ (as given in equation (4.3)). In conclusion, from the graphical representation of the step response for equations (4.2), we can deduce the amplitude $A = \frac{D}{D+k_0}\Delta C_{in}$, and the time response $t_r = \frac{3}{D+k_0}$. The parameters D and k_0 can therefore be readily calculated from the values of the amplitude $A = \frac{D}{D+k_0}\Delta C_{in}$ and the time response $t_r = \frac{3}{D+k_0}$.

Let us now consider that the measuring device for the reactant concentration gives a signal y which is proportional to the concentration C:

$$y = y_C C \tag{4.4}$$

And assume that the proportionality coefficient y_C is unknown, i.e. in this context y_C is an additional parameter. The step response of y will be very similar to that of C in Figure 4.1, except that the amplitude A is now equal to $y_C \frac{D}{D+k_0}\Delta C_{in}$. And it is now impossible to uniquely determine the values of the parameters y_C, D and k_0 from the values of the amplitude and time response of the step response. This means that the above three parameters are not identifiable. More precisely, only the following combinations of parameters are identifiable: $\theta_1 = D + k_0$ (from the time response $\frac{3}{D+k_0}$), and $\theta_2 = y_C D$ (= $A(D + k_0) = A\theta_1$).

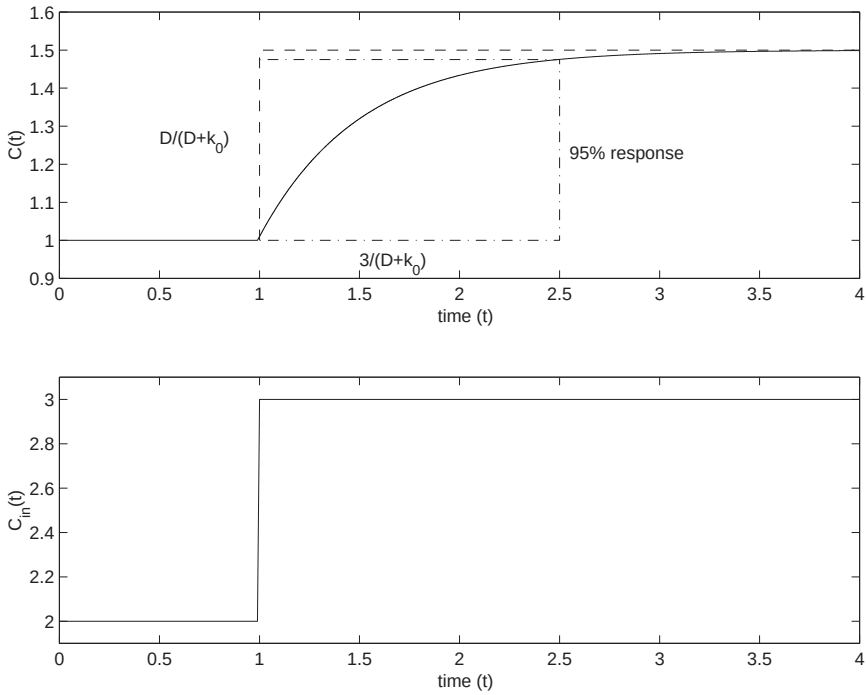

FIG. 4.1. Step response of a first order system.

4.3.2 *The Laplace Method*

The above simple example illustrates the structural identifiability of the two parameters k_0 and D, i.e. the structural (i.e. based on the model structure) property to determine uniquely the value of these parameters in "ideal" conditions. Conversely, the above example shows the lack of structural identifiability of the three parameters y_C, D and k_0, i.e. the structural property to determine non uniquely the value of these parameters in "ideal" conditions. Let us try to generalise the results of the above example.

Consider a dynamical model $\mathcal{M}(\theta)$ with the parametrisation $\theta = [\theta_1, \theta_2, ..., \theta_p]^T$, with q outputs y_i (i = 1 to q) (i.e. here measured variables) and m inputs u_j (j = 1 to m). If the model is linear, its dynamics will be described either by state space equations:

$$\frac{dx}{dt} = A(\theta)x(t,\theta) + B(\theta)u(t), \qquad x(0,\theta) = x_0(\theta) \tag{4.5}$$

$$y(t,\theta) = C(\theta)x(t,\theta) \tag{4.6}$$

or by a transfer function between the input vector $u(t)$ and the output vector $y(t,\theta)$, $H(s,\theta)$, which for the above state space model will be equal to:

$$H(s,\theta) = C(\theta)(sI - A(\theta))^{-1}B(\theta) \tag{4.7}$$

This transfer function for a SISO (Single Input Single Output) system is characterised by the ratio of two polynomials of order n (n is the number of states x), i.e. it has (maximum) $2n$ parameters.

Note that the transfer functions between C_{in} and C and, C_{in} and y for equations (4.2), (4.4) can be formally written as follows:

$$H(s) = \frac{b}{s+a} \tag{4.8}$$

with:

$$a = D + k_0 \tag{4.9}$$

$$b = D \text{ or } y_C D \tag{4.10}$$

In the above example, we note that D and k_0 can be readily obtained from the values of a and b in the first case ($D = b$, $k_0 = a - b$, while in the second case, it is not possible to distinguish between y_C and D from the value of b. This suggests the following possible generalisation. Indeed, the $2n$ parameters of the transfer function $H(s)$ are structurally identifiable from measurements of $y(t,\theta)$. The analysis can be based on the impulse response of this system, which is written as follows:

$$y(t,\theta) = \sum_{i=1}^{n} c_i(\theta) e^{\lambda_i(\theta)t} \tag{4.11}$$

The $2n$ coefficients c_i and λ_i can be determined from data of the output $y(t,\theta)$. Therefore the relationship between these coefficients and the transfer function parameters are very important for the structural identifiability. If we consider the Laplace transform for instance for n = 2 and distinct eigenvalues λ_i, we obtain:

$$H(s) = \frac{c_1}{s+\lambda_1} + \frac{c_2}{s+\lambda_2} \tag{4.12}$$

$$= \frac{(c_1+c_2)s - (c_2\lambda_1 + c_1\lambda_2)}{s^2 + (\lambda_1+\lambda_2)s + \lambda_1\lambda_2} \tag{4.13}$$

$$= \frac{\beta_1 s + \beta_2}{s^2 + \alpha_1 s + \alpha_2} \tag{4.14}$$

with $\beta_1 = c_1 + c_2$, $\beta_2 = -(c_2\lambda_1 + c_1\lambda_2)$, $\alpha_1 = \lambda_1 + \lambda_2$ and $\alpha_2 = \lambda_1\lambda_2$. This suggests that if there are no common factors in the numerator and denominator polynomials, the $2n$ parameters α_i and β_i can be determined from the output data just as the $2n$ coefficients c_i and λ_i.

Note that the identifiability property is typically valid for *almost* all parameter values: indeed for example there may be instances for which particular parameter

combinations or particular input functions give rise to pole-zero cancellation in the transfer function. However, these do not invalidate the general analysis (see also Section 4.3.4 here below).

4.3.3 *Some Generalisations and Definitions*

The above line of reasoning can lead to the following rather formal and abstract definitions of identifiability (see also [100] and [279] for a more rigourous treatment).

- *Definition # 1:* the parameter θ_i is structurally globally identifiable for the input class $\mathcal{U}$ if and only if for almost any value of the parameter vector θ (i.e. of the admissible parametric space $\mathcal{P}$) one has

$$\left.\begin{array}{l} \hat{\theta} \in \mathcal{P} \\ \hat{y}(\hat{\theta}, t) = y(\theta, t), \forall t > 0, \forall u \in \mathcal{U} \end{array}\right\} \Rightarrow \hat{\theta}_i = \theta_i$$

  i.e. in other words, the structurally global identifiability of the parameter θ_i means that if there exists another parameter vector $\hat{\theta}$ belonging to the admissible space for the parameters $\mathcal{P}$, and if the outputs y with both parameter vectors θ and $\hat{\theta}$ are equal for all time t and all input u, then the parameter θ_i (of the vector θ) and the parameter $\hat{\theta}_i$ (of the vector $\hat{\theta}$) are equal.
- *Definition # 2:* the parameter θ_i is structurally locally identifiable for the input class $\mathcal{U}$ if and only if for almost any value of the parameter vector θ (i.e. of the admissible parametric space $\mathcal{P}$) there exists a neighbourhood $V(\theta)$ such that

$$\left.\begin{array}{l} \hat{\theta} \in V(\theta) \subset \mathcal{P} \\ \hat{y}(\hat{\theta}, t) = y(\theta, t), \forall t > 0, \forall u \in \mathcal{U} \end{array}\right\} \Rightarrow \hat{\theta}_i = \theta_i$$

  The above definition is very similar to the preceding one, except that now we limit the domain of validity of the identifiability property to a subdomain (a neighbourhood $V(\theta)$) of the whole admissible space $\mathcal{P}$.
- *Definition # 3:* the model $\mathcal{M}(\theta)$ is structurally globally (locally) identifiable if and only if all the parameters θ_i are structurally globally (locally) identifiable.

The above definitions are obviously very important for defining the concept of structural identifiability, but they are not very useful for testing the identifiability of models. Before introducing different identifiability tests for nonlinear models, let us first further illustrate the concept via a second-order example in order to introduce some more generalisation (for state-space models).

4.3.4 *A Second-Order Example: The Two Interconnected CSTRs Model*

Let us go back to the model of the two interconnected CSTRs introduced in Chapter 2 (Section 2.5.1, Figure 2.12) with the following modifications:

1. S is the reactant of a first order chemical reaction;
2. S is fed in both tanks.

Let us note $F_{1,in}$, $S_{1,in}$, $F_{2,in}$, $S_{2,in}$ the influent flow rates and the influent concentration of S in tank 1 and 2, respectively, and k_0 the kinetic constant. The dynamics are then given by the following equations:

$$\frac{dS_1}{dt} = \frac{F_{1,in}}{V_1}S_{1,in} + \frac{F_1 - F_{1,in}}{V_1}S_2 - \frac{F_1}{V_1}S_1 - k_0 S_1 \tag{4.15}$$

$$\frac{dS_2}{dt} = \frac{F_{2,in}}{V_2}S_{2,in} + \frac{F_1}{V_2}S_1 - \frac{F_1 + F_{2,in}}{V_2}S_2 - k_0 S_2 \tag{4.16}$$

If we consider that the two influent concentrations are the process inputs, the above equations can be rewritten in the following matrix form:

$$\frac{d}{dt}\begin{pmatrix} S_1 \\ S_2 \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}\begin{pmatrix} S_1 \\ S_2 \end{pmatrix} + \begin{pmatrix} b_1 & 0 \\ 0 & b_2 \end{pmatrix}\begin{pmatrix} u_1 \\ u_2 \end{pmatrix} \tag{4.17}$$

with:

$$a_{11} = -\frac{F_1}{V_1} - k_0,\ a_{12} = \frac{F_1 - F_{1,in}}{V_1} \tag{4.18}$$

$$a_{21} = \frac{F_1}{V_2},\ a_{22} = -\frac{F_1 + F_{2,in}}{V_2} - k_0 \tag{4.19}$$

$$u_1 = S_{1,in},\ u_2 = S_{2,in} \tag{4.20}$$

$$b_1 = \frac{F_{1,in}}{V_1},\ b_2 = \frac{F_{2,in}}{V_2} \tag{4.21}$$

Assume now that the concentration of S can possibly be measured in both tanks, i.e.:

$$\begin{pmatrix} y_1 \\ y_2 \end{pmatrix} = \begin{pmatrix} c_1 & 0 \\ 0 & c_2 \end{pmatrix}\begin{pmatrix} S_1 \\ S_2 \end{pmatrix} \tag{4.22}$$

The transfer function *matrix* is readily derived by using the Laplace transform:

$$H(s) = \begin{pmatrix} \frac{Y_1(s)}{U_1(s)} & \frac{Y_1(s)}{U_2(s)} \\ \frac{Y_2(s)}{U_1(s)} & \frac{Y_2(s)}{U_2(s)} \end{pmatrix} \tag{4.23}$$

$$= \frac{1}{\Delta(s)}\begin{pmatrix} c_1 b_1 (s - a_{22}) & c_1 b_2 a_{12} \\ c_2 b_1 a_{21} & c_2 b_2 (s - a_{11}) \end{pmatrix} \tag{4.24}$$

with:

$$\Delta(s) = s^2 - (a_{11} + a_{22})s + a_{11}a_{22} - a_{12}a_{21} \tag{4.25}$$

Let us now examine the identifiability for different situations.

$u_1 \neq 0$, $u_2 = 0$, and only y_1 measured. The transfer function $\frac{Y_1(s)}{U_1(s)}$ can be written in the format (4.14) with:

$$\beta_1 = c_1 b_1, \ \beta_2 = -c_1 b_1 a_{22} \tag{4.26}$$

$$\alpha_1 = -(a_{11} + a_{22}), \ \alpha_2 = a_{11} a_{22} - a_{12} a_{21} \tag{4.27}$$

From the structural identifiability of α_1, α_2, β_1 and β_2, and the above relationships, we can readily conclude that only four parameter combinations out of the six parameters of $\frac{Y_1(s)}{U_1(s)}$, i.e. $c_1 b_1$, a_{22}, a_{11} and $a_{12} a_{21}$ are identifiable. In other words, the six parameters a_{11}, a_{22}, a_{12}, a_{21}, c_1 and b_1 are *not* identifiable while the four "parameters" $\theta_1 = c_1 b_1$, $\theta_2 = a_{22}$, $\theta_3 = a_{11}$ and $\theta_4 = a_{12} a_{21}$ are:

$$\alpha_1, \ \alpha_2, \ \beta_1, \ \beta_2 \ \Rightarrow \begin{cases} \theta_1 = \beta_1 \\ \theta_2 = -\frac{\beta_2}{\beta_1} \\ \theta_3 = -\alpha_1 + \frac{\beta_2}{\beta_1} \\ \theta_4 = -\alpha_2 - \frac{\beta_2}{\beta_1}(-\alpha_1 + \frac{\beta_2}{\beta_1}) \end{cases}$$

Now, can we expect to have more identifiable parameters if we consider the second input and/or the second output?

$u_1 \neq 0$, $u_2 = 0$, y_1 and y_2 measured. We have here an additional measurement. However the denominators of $\frac{Y_1(s)}{U_1(s)}$ and $\frac{Y_2(s)}{U_1(s)}$ are the same. So any improvement in the number of identifiable parameters may only come from the numerator of $\frac{Y_2(s)}{U_1(s)}$, i.e. $b_1 c_2 a_{12}$. We note that a priori nothing is gained (we have one more parameter c_2 and one more identifiable parameter combination $b_1 c_2 a_{12}$) except if there is some a priori information about c_2. A priori information may be, for instance, the equality between c_1 and c_2 ($c_1 = c_2$) (this case is even the most probable in our example), then the numerator of $\frac{Y_2(s)}{U_1(s)}$ is equal to $b_1 c_1 a_{12}$. Since $b_1 c_1$ is already identifiable (see the preceding case), then a_{12} is identifiable. Now, since a_{11} and a_{22} are also identifiable (see above), then we can deduce from the definition of α (4.27) that a_{12} is also identifiable.

$u_1 \neq 0$, $u_2 \neq 0$, and only y_1 measured. Let us evaluate what is gained by considering both inputs u_1 and u_2 (in practice via test-inputs applied at different time instants so that both responses can be completely distinguished from each other, so that $u_1(t) \neq \gamma u_2(t)$ at each time time instant). A conclusion similar to the one of the previous paragraph can be drawn, i.e. there is one extra parameter (b_2) and one extra parameter combination ($c_1 b_2 a_{12}$), and nothing is gained in terms of identifiability, except if there is some a priori information about b_2.

Now, if we consider the particular case when the same inputs are applied at the same time (i.e. $u_1 = u_2$), then the transfer function $\frac{Y_1(s)}{U_1(s)}$ is equal to:

$$\frac{Y_1(s)}{U_1(s)} = \frac{c_1 b_1 s + c_1(b_2 a_{12} - b_1 a_{22})}{\Delta(s)} \tag{4.28}$$

We are then in the particular situation when some input combinations may lead to a loss of identifiability (only 4 (instead of 5) parameters are then identifiable). This illustrates the introduction of the term *for almost any value of the parameters* in the above definitions, and also points to the fact that the choice of appropriate experimental conditions can have a great influence on the *practical* identifiability of the system (but this is the topic of Chapter 5).

$u_1 \neq 0$, $u_2 = 0$, only y_2 measured, and $a_{12} = 0$. Let us finally consider the case when $a_{12} = 0$, i.e. when we consider a sequence of two CSTRs without any possible flow back from tank 2 to tank 1 (i.e. $F_2 = 0$, or more precisely $F_1 = F_{1,in}$, see also Figure 2.12). Then the transfer function $\frac{Y_2(s)}{U_1(s)}$ is equal to:

$$\frac{Y_2(s)}{U_1(s)} = \frac{c_2 b_1 a_{21}}{(s - a_{11})(s - a_{22})} \tag{4.29}$$

The parameters a_{11} and a_{22} are identifiable, but only locally. Indeed it is not possible to distinguish between both parameters, since they have both two possible (interchangeable) values for the above configuration. In other words, this means that in the present example, we cannot distinguish between the two volumes V_1 and V_2, i.e. we cannot say if the first volume is small and the second is large or the reverse.

4.4 Methods for Testing Structural Identifiability of Nonlinear Systems

In this section, we introduce different approaches to test the structural identifiability of models. These represent the most largely used methods for structural identifiability. But the list is not exhaustive. Our objective is indeed to provide tools that can be helpful for testing structural identifiability, not to write a full monograph on the topic (see e.g. [100], [279], [254] for a more rigourous approach and technical mathematical details). Note that, in addition to the methods discussed in this section, we shall consider another method illustrated with the first example (Monod model) in the next section dedicated to illustrative examples.

The Laplace transform method has already been introduced in Section 4.2. One of the most important difficulties with the Laplace transform is that strictly speaking it only applies to linear models, and that results obtained from linearised models of nonlinear models may be difficult to interpret. The results obtained for the linearised model are only sufficient conditions: this means that the (combinations of) parameters that are identifiable for the model linearised around some steady state are also identifiable for the nonlinear model around that steady state only.

The basic concepts of each method are first presented. Their application is then illustrated on a simple example (nonlinear model linear in the parameters) in Section 4.4.5.

4.4.1 *Taylor Series Expansion*

Consider that the model equations are written under the following form:

$$\frac{dx}{dt} = f(x, u, \theta), \quad x(0) = x_0(\theta) \tag{4.30}$$

$$y(t, \theta) = h(x, \theta) \tag{4.31}$$

where x, u, y and θ represent the state vector, the input vector, the output (measured variable) vector, and the (unknown) parameter vector, respectively.

The method is based on a Taylor series expansion of the observations y(t) around time t=0:

$$y(t) = y(0) + t\frac{dy}{dt}(0) + \frac{t^2}{2!}\frac{d^2y}{dt^2}(0) + ... \tag{4.32}$$

and consists of looking at the successive derivatives to check if they contain information about the parameters to be identified. More precisely, $y(0)$ and the successive derivatives of $y(t)$ at time t = 0 can be expressed from the model equations (4.30)(4.31) as functions of the unknown parameters $\theta^T = [\theta_1, \theta_2, ..., \theta_p]$:

$$y(0) = \gamma_0(\theta) \tag{4.33}$$

$$\frac{dy}{dt}(0) = \gamma_1(\theta) \tag{4.34}$$

$$\vdots \tag{4.35}$$

$$\frac{d^q y}{dt^q}(0) = \gamma_q(\theta) \tag{4.36}$$

The second step consists of trying to invert the above expressions (4.33)-(4.36) so as to express the parameters θ_i (i = 1 to p) as functions of only $y(0)$, its successive derivatives and the input u, i.e.:

$$\theta_1 = \beta_1(y(0), \frac{dy}{dt}(0), ..., \frac{d^q y}{dt^q}(0), u) \tag{4.37}$$

$$\theta_2 = \beta_2(y(0), \frac{dy}{dt}(0), ..., \frac{d^q y}{dt^q}(0), u) \tag{4.38}$$

$$\vdots \tag{4.39}$$

$$\theta_p = \beta_p(y(0), \frac{dy}{dt}(0), ..., \frac{d^q y}{dt^q}(0), u) \tag{4.40}$$

If such a set of equations exist, this means that the parameters θ_i (i = 1 to p) are structurally identifiable. But it may also happen that the above set of equations can be written only for combinations of the parameters θ_i, and then only these combinations are identifiable.

Note that generally speaking the number of successive derivatives (= q) used for the identifiability analysis is not equal to the number of unknown parameters (= p). q is typically at least equal to p (because at least p relations are needed to invert and obtain expressions for the p parameters θ_i), but it may be larger in order to obtain new expressions that are independent of the preceding ones and therefore susceptible to introduce new information for the analysis.

Remark: for simplicity, we had chosen one time instant (t = 0) in the expansion (4.32). But other time instants may be used because these can be helpful in order to simplify the analysis by considering different (simpler) submodels (see e.g. the example in Section 4.6.3).

4.4.2 *Generating Series*

The Generating Series method is based on nonlinear control theory concepts, basically on the Lie derivatives and its link to observability of nonlinear systems. Without entering into the details, the method can be briefly summarised as follows:

Let us consider that the system equations can be written as follows[4]:

$$\frac{dx}{dt} = f_0(x,\theta) + \sum_{i=1}^{m} u_i(t)\, f_i(x,\theta), \quad x(0) = x_0(\theta) \tag{4.41}$$

$$y(t,\theta) = h(x,\theta) \tag{4.42}$$

The analysis is based on the output functions $h(x,\theta)$ and its successive Lie derivatives $L_{f_{j0}} \ldots L_{f_{jk}} h(x,\theta)$ evaluated at t=0. The Lie derivative along the vector field f_i is defined as follows:

$$L_{f_i} = \sum_{j=1}^{n} f_{j,i}(x,\theta) \frac{\partial}{\partial x_j} \tag{4.43}$$

with $f_{j,i}$ the j^{th} component of f_i. As a matter of illustration, the Lie derivative of h and of L_{f_k} along the vector field f_i are equal to:

$$L_{f_i} h(x,\theta) = \sum_{j=1}^{n} f_{j,i}(x,\theta) \frac{\partial}{\partial x_j} h(x,\theta) \tag{4.44}$$

$$L_{f_i} L_{f_k} = \sum_{j=1}^{n} f_{j,i}(x,\theta) \frac{\partial}{\partial x_j} L_{f_k} \tag{4.45}$$

Similarly to the Taylor series expansion method, we look at the successive generated Lie derivatives evaluated at time t = 0 (and assumed "known") to check if they contain information about the parameters to be identified.

[4]Note that the model (4.41) is linear in $u(t)$.

4.4.3 *Local State Isomorphism*

Let us consider that the system equations can be written as follows (it is only for convenience of the presentation of the method that we consider here a state representation different from the above equation (4.41)):

$$\frac{dx}{dt} = f(x,\theta) + u^T(t)g(x,\theta), \quad x(0) = x_0(\theta) \tag{4.46}$$

$$y(t,\theta) = h(x,\theta) \tag{4.47}$$

Let us denote $\bar{x}$ and $\tilde{x}$ two states corresponding to two different sets of parameter values, $\bar{\theta}$ and $\tilde{\theta}$ respectively. The models corresponding to each parameter set will have the same input-output behaviour (and $\bar{\theta}$ and $\tilde{\theta}$ are therefore not distinguishable) for any input u up to a time $t_1 > 0$ if and only if there exists a local state isomorphism:

$$\lambda : V \to R^n, \bar{x} \to \tilde{x} = \lambda(\bar{x}), \ V \text{is an open neighbourhood of } \bar{x}(0) \tag{4.48}$$

such that for any $\bar{x}$ in the neighbourhood $V(\bar{x}(0))$ the following conditions are satisfied:

$$\lambda \text{ is a diffeomorphism} : rank\frac{\partial\lambda(x=\bar{x})}{\partial x^T} = n \tag{4.49}$$

$$\text{the initial states correspond} : \lambda(\bar{x}(0)) = \tilde{x}(0) \tag{4.50}$$

$$\text{the drift terms correspond} : f(\lambda(\bar{x}),\tilde{\theta}) = \frac{\partial\lambda(x=\bar{x})}{\partial x^T} f(\lambda(\bar{x}),\bar{\theta}) \tag{4.51}$$

$$\text{the input terms correspond} : g(\lambda(\bar{x}),\tilde{\theta}) = \frac{\partial\lambda(x=\bar{x})}{\partial x^T} g(\lambda(\bar{x}),\bar{\theta}) \tag{4.52}$$

$$\text{the observations correspond} : h(\lambda(\bar{x}),\tilde{\theta}) = h(\bar{x},\bar{\theta}) \tag{4.53}$$

One can test the structural identifiability by looking at all the solutions for $\bar{\theta}$ and λ of the above equations (4.49)-(4.53). If for almost any $\tilde{\theta}$ the only possible solution is $\bar{\theta} = \tilde{\theta}$, $\lambda(\bar{x}) = \tilde{x}$, then the model is uniquely identifiable.

Remark: make sure that you don't confuse between the two different notations, e.g. $\tilde{\theta}$ and $\bar{\theta}$, or $\tilde{x}$ and $\bar{x}$!

4.4.4 *Transformation of Nonlinear Models*

Another way to analyse the structural identifiability is to transform the nonlinear model into a model linear in the parameters, and then look at the identifiability of the linear model. A better understanding of the approach can be drawn from the examples here below in Sections 4.4.5 and 4.6.3.

4.4.5 *A Simple Example*

Let us illustrate the above identifiability tests with a simple nonlinear model linear in three parameters ($\theta_1, \theta_2, \theta_3$):

$$\frac{dx_1}{dt} = -\theta_1 x_1 - \theta_2 x_1 + \theta_3 x_1 x_2 + u,\ x_1(0) = 1 \tag{4.54}$$

$$\frac{dx_2}{dt} = \theta_2 x_1 - \theta_3 x_1 x_2,\ x_2(0) = 0 \tag{4.55}$$

$$y(t, \theta) = x_1 \tag{4.56}$$

This model holds for instance for a bioprocess in a batch reactor where x_2 is the concentration of a reactant and x_1 the concentration of an autocatalyst (e.g. microorganisms), and where there are three reactions: autocatalysis of x_1 with first order kinetics with respect to the reactant ($r_1 = \theta_3 x_1 x_2$), a decomposition (e.g. lysis) of x_1 into x_2 ($r_2 = \theta_2 x_1$) and a third reaction which may be a mortality reaction of x_1 ($r_3 = \theta_1 x_1$). Beside y, the input u is assumed to be known (in the context of the above example, u could be interpreted as an external addition of the autocatalyst x_1 in quantities small enough to keep the variations of the fermenter volume negligible).

The choice of the above example is motivated by our will to give an illustration of the above identifiability analysis tools that remains as simple and clear as possible. This has led to the present choice, i.e. a model linear in the parameters and nonlinear in the state variables, yet rather simple and with a potential connection with models considered in WWTP. This choice was a difficult one for us, because we are conscious that the linearity in the parameters may be interpreted as a limitation of the applicability of the results presented here. But starting with a nonlinear example would have led us to considerations that may have hidden the basic remarks that we feel are necessary to be understood to apply the proposed analysis tools. Other examples of models nonlinear in the parameters are therefore dealt with in the rest of the chapter, more precisely in Section 4.6.

Taylor series expansion. Let us start with the Taylor series expansion of $y(t, \theta)$:

$$\frac{dy}{dt}(0) = -(\theta_1 + \theta_2) + u \tag{4.57}$$

$$\frac{d^2y}{dt^2}(0) = (\theta_1 + \theta_2)^2 - (\theta_1 + \theta_2)u + \theta_2\theta_3 \tag{4.58}$$

$$\frac{d^3y}{dt^3}(0) = -(\theta_1 + \theta_2)^3 + (\theta_1 + \theta_2)^2 u + 2\theta_2\theta_3 u - 2\theta_2\theta_3(\theta_1 + \theta_2) - \theta_2\theta_3^2 \tag{4.59}$$

Let us denote:

$$z_i = \frac{d^i y}{dt^i}(0) \tag{4.60}$$

Basically the z_i can be considered to be variables that have a known values since they can be readily obtained from (ideal) measurements y. The above equations can be rewritten as follows:

$$z_1 = -(\theta_1 + \theta_2) + u \tag{4.61}$$

$$z_2 = (\theta_1 + \theta_2)^2 - (\theta_1 + \theta_2)u + \theta_2\theta_3 \tag{4.62}$$

$$z_3 = -(\theta_1 + \theta_2)^3 + (\theta_1 + \theta_2)^2 u + 2\theta_2\theta_3 u - 2\theta_2\theta_3(\theta_1 + \theta_2) - \theta_2\theta_3^2 \tag{4.63}$$

We have three unknown parameters θ_1, θ_2, θ_3 and three equations in z_i (i = 1, 2, 3). The difficult task can now start, i.e. to see if it is possible to invert the above equations (4.61) (4.62) (4.63) to obtain expressions for the θ_i that are only functions of the z_i and of the input u. One possible way to proceed is the following. A look at the above equations shows that there are three groups of parameters: $\theta_1 + \theta_2$, $\theta_2\theta_3$ (last term of the second equation), and $\theta_2\theta_3^2$ (last term of the third equation). $\theta_1 + \theta_2$ can be expressed as a function of z_1 and u from the first equation (4.61). The second equation (4.62) can be used to express $\theta_2\theta_3$ as a function of z_2, u and z_1 (via $\theta_1 + \theta_2$) only. Finally the same procedure can be followed in the third equation (4.63) to express $\theta_2\theta_3^2$ as a function of z_1, z_2, z_3 and u:

$$\theta_1 + \theta_2 = u - z_1 \tag{4.64}$$

$$\theta_2\theta_3 = z_2 - (u - z_1)^2 + (u - z_1)u \tag{4.65}$$

$$\theta_2\theta_3^2 = z_3 + (u - z_1)^3 + (u - z_1)^2 u - 2(u - (u - z_1))(z_2 - (u - z_1)^2 + (u - z_1)u) \tag{4.66}$$

It is now possible to compute θ_3 (as the ratio of $\theta_2\theta_3^2$ and $\theta_2\theta_3$), θ_2 and θ_1, successively:

$$\theta_1 = u - z_1 - (z_2 - 2u^2 + z_1^2 + 3uz_1)^2(2u^3 - 10u^2 z_1 + 6uz_1^2 + z_1^3 + 2z_1 z_2 - z_3) \tag{4.67}$$

$$\theta_2 = (z_2 - 2u^2 + z_1^2 + 3uz_1)^2(2u^3 - 10u^2 z_1 + 6uz_1^2 + z_1^3 + 2z_1 z_2 - z_3) \tag{4.68}$$

$$\theta_3 = \frac{2u^3 - 10u^2 z_1 + 6uz_1^2 + z_1^3 + 2z_1 z_2 - z_3}{z_2 - 2u^2 + z_1^2 + 3uz_1} \tag{4.69}$$

This shows that the three parameters θ_1, θ_2 and θ_3 can be formally computed from the values of z_1, z_2, z_3, and u, i.e. they are structurally identifiable.

Generating series. Let us now look at the generating series test. We know that:

$$f_0 = \begin{pmatrix} -\theta_1 x_1 - \theta_2 x_1 + \theta_3 x_1 x_2 \\ \theta_2 x_1 - \theta_3 x_1 x_2 \end{pmatrix}, \quad f_1 = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad h = x_1 \tag{4.70}$$

The Lie derivatives are equal to:

$$L_{f_0} = [-\theta_1 x_1 - \theta_2 x_1 + \theta_3 x_1 x_2]\frac{\partial}{\partial x_1} + [\theta_2 x_1 - \theta_3 x_1 x_2]\frac{\partial}{\partial x_2} \tag{4.71}$$

$$L_{f_1} = \frac{\partial}{\partial x_1} \tag{4.72}$$

Let us consider the following three successive Lie derivatives of h evaluated at time t = 0 (some others could have been written down too, e.g. $L_{f_1} L_{f_0} h(0)$ $(= \theta_3 x_1(0) = 0)$, but are unnecessary here):

$$z_1 = L_{f_0} h(0) = -\theta_1 - \theta_2 \tag{4.73}$$

$$z_2 = L_{f_0} L_{f_0} h(0) = (\theta_1 + \theta_2)^2 + \theta_2\theta_3 \tag{4.74}$$

$$\begin{aligned} z_3 &= L_{f_0} L_{f_0} L_{f_0} h(0) \\ &= -(\theta_1 + \theta_2)[(\theta_1 + \theta_2)^2 + 2\theta_2\theta_3 - \theta_2\theta_3(\theta_1 + \theta_2) - \theta_2\theta_3^2] \end{aligned} \tag{4.75}$$

By using the same argument as for the Taylor series approach (inversion of the expressions), we can rewrite the three parameters in terms of the successive Lie derivatives, and therefore the three parameters θ_1, θ_2 and θ_3 are structurally identifiable.

Note the resemblance of the above expressions (4.73)-(4.75) with those obtained with the Taylor series expansion approach (4.61)-(4.63), but without the terms in the input u. The present approach allows the separation of both types of terms, i.e. the terms depending on the state variables and those depending on the inputs u. This offers the advantage of handling possibly simpler expressions (compare equations (4.73)-(4.75) and (4.61)-(4.63)).

Local state isomorphism. Let us now apply the local state isomorphism. Now we have:

$$f = \begin{pmatrix} -\theta_1 x_1 - \theta_2 x_1 + \theta_3 x_1 x_2 \\ \theta_2 x_1 - \theta_3 x_1 x_2 \end{pmatrix}, \; g = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \; h = x_1 \tag{4.76}$$

First note that the dimension of λ is 2:

$$\lambda = \begin{pmatrix} \lambda_1 \\ \lambda_2 \end{pmatrix} \tag{4.77}$$

i.e.:

$$\begin{pmatrix} \tilde{x}_1 \\ \tilde{x}_2 \end{pmatrix} = \begin{pmatrix} \lambda_1(\bar{x}_1, \bar{x}_2) \\ \lambda_2(\bar{x}_1, \bar{x}_2) \end{pmatrix} \tag{4.78}$$

From the relations (4.48), we have here:

$$\tilde{x}_1 = \lambda_1(\bar{x}_1) \tag{4.79}$$

And the condition (4.53) gives:

$$\lambda_1(\bar{x}_1) = \bar{x}_1 \tag{4.80}$$

This gives:

$$\tilde{x}_1 = \lambda_1(\bar{x}_1) = \bar{x}_1 \tag{4.81}$$

This means that:

$$\frac{\partial \lambda_1}{\partial x_1} = 1, \ \frac{\partial \lambda_1}{\partial x_2} = 0 \tag{4.82}$$

If we consider now the relation (4.52), we have:

$$\begin{pmatrix} 1 \\ 0 \end{pmatrix} = \frac{\partial \lambda}{\partial x^T} \begin{pmatrix} 1 \\ 0 \end{pmatrix} = \begin{pmatrix} \frac{\partial \lambda_1}{\partial x_1} \\ \frac{\partial \lambda_2}{\partial x_1} \end{pmatrix} \tag{4.83}$$

This implies that:

$$\frac{\partial \lambda_2}{\partial x_1} = 0 \tag{4.84}$$

Let us now look at the relation (4.51), which specialises here as follows:

$$\begin{pmatrix} -\tilde{\theta}_1\tilde{x}_1 - \tilde{\theta}_2\tilde{x}_1 + \tilde{\theta}_3\tilde{x}_1\tilde{x}_2 \\ \tilde{\theta}_2\tilde{x}_1 - \tilde{\theta}_3\tilde{x}_1\tilde{x}_2 \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & \frac{\partial \lambda_2}{\partial x_2} \end{pmatrix} \begin{pmatrix} -\bar{\theta}_1\bar{x}_1 - \bar{\theta}_2\bar{x}_1 + \bar{\theta}_3\bar{x}_1\bar{x}_2 \\ \bar{\theta}_2\bar{x}_1 - \bar{\theta}_3\bar{x}_1\bar{x}_2 \end{pmatrix} \tag{4.85}$$

Since $\tilde{x}_1 = \bar{x}_1$ and $\tilde{x}_2 = \lambda_2(\bar{x})$, the first row implies that:

$$-\tilde{\theta}_1\bar{x}_1 - \tilde{\theta}_2\bar{x}_1 + \tilde{\theta}_3\bar{x}_1\lambda_2(\bar{x}) = -\bar{\theta}_1\bar{x}_1 - \bar{\theta}_2\bar{x}_1 + \bar{\theta}_3\bar{x}_1\bar{x}_2 \tag{4.86}$$

since $\bar{x}_1$ and $\bar{x}_2$ are independent (they are solutions of the two ordinary differential equations (4.54)(4.55)), we have:

$$\tilde{\theta}_1 + \tilde{\theta}_2 = \bar{\theta}_1 + \bar{\theta}_2 \tag{4.87}$$

$$\lambda_2(\bar{x}) = \frac{\bar{\theta}_3}{\tilde{\theta}_3}\bar{x}_2 \tag{4.88}$$

Therefore, the derivative of λ_2 with respect to x_2 is equal to:

$$\frac{\partial \lambda_2}{\partial x_2} = \frac{\bar{\theta}_3}{\tilde{\theta}_3} \tag{4.89}$$

The second row of (4.85) then becomes:

$$\tilde{\theta}_2\bar{x}_1 - \bar{\theta}_3\bar{x}_1\bar{x}_2 = \bar{\theta}_2\frac{\bar{\theta}_3}{\tilde{\theta}_3}\bar{x}_1 - \bar{\theta}_3\frac{\bar{\theta}_3}{\tilde{\theta}_3}\bar{x}_1\bar{x}_2 \tag{4.90}$$

which implies that:

$$\tilde{\theta}_2\tilde{\theta}_3 = \bar{\theta}_2\bar{\theta}_3, \ \tilde{\theta}_3 = \bar{\theta}_3 \tag{4.91}$$

The condition (4.50) is immediate, and the relation (4.49) simply implies that $\tilde{\theta}_3$ must be different from zero.

We can then conclude, specifically from (4.87) and (4.91) that the three parameters θ_1, θ_2 and θ_3 are here also structurally identifiable.

Transformation of nonlinear models. The objective of the method is to rewrite the model in input-output format linear in the parameters. This is performed here by the elimination of x_2 via differentiating the output x_1 twice.

x_2 can be put in evidence from equation (4.54):

$$x_2 = \frac{1}{\theta_3 x_1}(\frac{dx_1}{dt} + \theta_1 x_1 + \theta_2 x_1 - u) \tag{4.92}$$

By differentiating the output y (= x_1) twice with respect to time t, we obtain:

$$\frac{d^2y}{dt^2} - \frac{1}{y}(\frac{dy}{dt})^2 + \frac{u}{y} = (\theta_1+\theta_2)\frac{dy}{dt}(\frac{dy}{dt}-1) + \theta_2\theta_3 y^2 - \theta_3 y(\frac{dy}{dt}-u) - \theta_3(\theta_1+\theta_2)y\frac{dy}{dt} \tag{4.93}$$

It can be rewritten in the usual linear regression format $Y = \theta^T \Phi$ with:

$$Y = \frac{d^2y}{dt^2} - \frac{1}{y}(\frac{dy}{dt})^2 + \frac{u}{y},\ \theta = \begin{pmatrix} \theta_1 + \theta_2 \\ \theta_2\theta_3 \\ \theta_3 \\ \theta_3(\theta_1+\theta_2) \end{pmatrix},\ \Phi = \begin{pmatrix} \frac{dy}{dt}(\frac{dy}{dt}-1) \\ y^2 \\ y(\frac{dy}{dt}-u) \\ y\frac{dy}{dt} \end{pmatrix} \tag{4.94}$$

θ is identifiable if the components of the regressor vector Φ are independent (see e.g. [14] [165] for more details). It is then obvious that it is structurally possible to reconstruct the parameters θ_1, θ_2 and θ_3 from the parameter vector θ.

4.5 The Lyapunov-Based Method: An Historical Perspective with the Monod Model

The Monod model is largely used in biotechnological process applications, and in particular in biological wastewater treatment, to characterise growth kinetics. It has been the object of many (structural and practical) identifiability studies since the seventies (see e.g. [2], [126], [276], [100], [161]). In this section we present the first structural identifiability analysis performed on the Monod model. It has been published by Aborhey and Williamson in 1978 ([2]). The original aspect of the proposed analysis is that it is based on a Lyapunov function, a concept largely used to analyse the stability of dynamical systems. In that sense, this approach can be viewed as another method to analyse the structural identifiability, although it is not very popular so far.

Let us start by rewriting the mass balance equations of a simple microbial growth process with Monod kinetics in a CSTR:

$$\frac{dX}{dt} = \frac{\mu_{max} S X}{K_S + S} - DX \tag{4.95}$$

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_{max} S X}{K_S + S} + DS_{in} - DS \tag{4.96}$$

In the above model, there are 3 parameters: μ_{max}, K_S, and Y.

Assume now that X and S are accessible for on-line measurement, as well as D and S_{in}. The structural identifiability of the 3 parameters can be deduced from the existence of an estimation algorithm that is shown to be theoretically convergent.

In their paper, Aborhey and Williamson ([2]) propose the following estimation scheme[5]:

$$\frac{dz_1}{dt} = X[\hat{\mu} - D - g_1(z_1 - X)] \tag{4.97}$$

$$\frac{dz_2}{dt} = X[\hat{\alpha} - g_2(z_2 - S)] + DS_{in} - DS \tag{4.98}$$

$$\frac{d\hat{\mu}_{max}}{dt} = -\lambda_1 XS(z_1 - X) \tag{4.99}$$

$$\frac{d\hat{K}_S}{dt} = X[\lambda_2\hat{\mu}(z_1 - X) + \lambda_3\hat{\alpha}(z_2 - S)] \tag{4.100}$$

$$\frac{d\hat{\alpha}_m}{dt} = -\lambda_4 XS(z_2 - S) \tag{4.101}$$

with:

$$\alpha_m = -\frac{\mu_{max}}{Y},\ \alpha = -\frac{\mu}{Y},\ \hat{\mu} = \frac{\hat{\mu}_{max}S}{\hat{K}_S + S} \tag{4.102}$$

Let us choose the estimator design parameters g_i (i = 1, 2) and λ_i (i = 1 to 4) such that:

$$g_i > \frac{DS_{in}}{2(S + K_S)X} \tag{4.103}$$

$$\lambda_i > 0 \tag{4.104}$$

It is obvious that the estimate of Y is readily derived from the estimates of μ_{max} and α_m:

$$\hat{Y} = \frac{\hat{\mu}_{max}}{\hat{\alpha}_m} \tag{4.105}$$

Let us now consider the following Lyapunov (positive definite: $V > 0$) candidate function:

$$V = (S + K_S)(l_1 e_1^2 + l_2 e_2^2) + \sum_{i=3}^{5} l_i e_i^2,\ l_i > 0, \quad \text{for i = 1 to 5} \tag{4.106}$$

where e_i are the following error terms:

$$e_1 = z_1 - X \tag{4.107}$$

[5]As the reader may detect either by personal experience or by looking at the chapter on state observation (Chapter 7), the following structure resembles somewhat to that of a classical observer like the extended Luenberger observer, but the choice of the observer gains is different (it is based on a Lyapunov function).

$$e_2 = z_2 - S \tag{4.108}$$
$$e_3 = \hat{\mu}_{max} - \mu_{max} \tag{4.109}$$
$$e_4 = \hat{K}_S - K_S \tag{4.110}$$
$$e_5 = \hat{\alpha}_m - \alpha \tag{4.111}$$

The estimation algorithm (4.97)-(4.101) will be convergent, i.e. the estimates of μ_{max}, K_S, and Y will converge to their true values, if the time derivative of V along the solutions of (4.95)(4.96)(4.97)-(4.101) is negative definite. It is what we are going to check in the following paragraphs.

Let us first write the dynamics of the error terms e_i (i = 1 to 5) from (4.95), (4.96), (4.97)-(4.101), (4.107)-(4.111). This gives:

$$\frac{de_1}{dt} = -g_1 X e_1 - X(\hat{\mu} - \mu) \tag{4.112}$$
$$\frac{de_2}{dt} = -g_2 X e_2 - X(\hat{\mu} - \mu) \tag{4.113}$$
$$\frac{de_3}{dt} = -\lambda_1 X S e_1 \tag{4.114}$$
$$\frac{de_4}{dt} = X[\lambda_2 \hat{\mu} e_1 + \lambda_3 \hat{\alpha} e_2] \tag{4.115}$$
$$\frac{de_5}{dt} = -\lambda_4 X S e_2 \tag{4.116}$$

Let us compute dV/dt:

$$\begin{aligned}\frac{dV}{dt} = {} & l_1 e_1^2 [\frac{dS}{dt} - 2g_1(S + K_S)X] + l_2 e_2^2 [\frac{dS}{dt} - 2g_2(S + K_S)X] \\ & + 2[e_1 e_3 (l_1 - l_3\lambda_1) X S + e_1 e_4 (l_4\lambda_2 - l_1) X \\ & + e_2 e_4 (l_4\lambda_3 - l_2) X + e_2 e_5 (l_2 - l_5\lambda_4) X S]\end{aligned} \tag{4.117}$$

Now we choose l_i (i = 1 to 5) such that:

$$l_1 = l_3\lambda_1,\ l_4\lambda_2 = l_1,\ l_4\lambda_3 = l_2,\ l_2 = l_5\lambda_4 \tag{4.118}$$

Then the last four terms of dV/dt are equal to zero, i.e.:

$$\frac{dV}{dt} = l_1 e_1^2 [\frac{dS}{dt} - 2g_1(S + K_S)X] + l_2 e_2^2 [\frac{dS}{dt} - 2g_2(S + K_S)X] \tag{4.119}$$

By using (4.103), it is straighforward that dV/dt is negative, since $dS/dt \leq DS_{in}$. And if $dV/dt = 0$ for some time t between t_1 and t_2, this means that:

$$e_1 = e_2 = 0 \text{ and } \frac{de_1}{dt} = \frac{de_2}{dt} = 0 \tag{4.120}$$

Let us introduce the above equalities (4.120) in equations (4.112) and (4.113). Let us only consider the calculations with equation (4.112) here (the argument

is completely similar with (4.113)). $e_1 = 0$ and $de_1/dt = 0$ imply that equation (4.112) becomes:

$$\frac{\mu_{max} S}{K_S + S} = \frac{\hat{\mu}_{max} S}{\hat{K}_S + S} \tag{4.121}$$

Let us multiply both sides of the above equation by $(K_S + S)$ and then substract (also on both sides) $\hat{\mu}_{max} S$.

We obtain (after changing signs on both sides):

$$\hat{\mu}_{max} S - \mu_{max} S = \hat{\mu}_{max} S - \frac{\hat{\mu}_{max} S(K_S + S)}{\hat{K}_S + S} \tag{4.122}$$

If we multiply and divide the first term of the right hand side by $(\hat{K}_S + S)$, the right hand side becomes:

$$\frac{\hat{\mu}_{max} S}{\hat{K}_S + S}(\hat{K}_S - K_S) \tag{4.123}$$

Thus equation (4.122) becomes:

$$e_3 S = \hat{\mu} e_4 \tag{4.124}$$

Similarly we obtain from equation (4.113):

$$e_5 S = \hat{\alpha} e_4 \tag{4.125}$$

For the last three equations (4.114)(4.115)(4.116), we readily obtain by using equation (4.120):

$$\frac{de_3}{dt} = \frac{de_4}{dt} = \frac{de_5}{dt} = 0 \tag{4.126}$$

Since S, $\hat{\mu}$ and $\hat{\alpha}$ will generally speaking be varying independently from each other, this implies that:

$$e_3 = e_4 = e_5 = 0 \tag{4.127}$$

on the interval $[t_1,\ t_2]$ provided that $S(t)$ is not constant on this interval.

Therefore the time derivative of the candidate Lyapunov function V is negative definite. This implies the convergence of the estimation algorithm, and in consequence, the identifiability of the parameters of the Monod model. Or in other words, if we are able to build an estimation algorithm that is mathematically guaranteed to give estimates that converge to their true values, this means that a fortiori, the parameters that are considered in the estimator are structurally identifiable.

4.6 Example #2: Respirometer-based Models

Let us perform the analysis of the structural identifiability of the four models introduced in Chapter 3 (Section 3.2) based on the on-line measurement of the oxygen uptake rate via a respirometer (see also [74]). We shall consider the following approaches for the different models:

1. first order kinetics (type 1): Laplace transform;
2. single Monod model (type 2): Taylor series expansion;
3. double Monod model (type 3): nonlinear transformation;
4. modified ASM1 model (type 4): nonlinear transformation and generating series.

4.6.1 *Identifiability of the First Order Kinetics Model (Laplace Transform)*

Recall that the equations of the model are the following:

$$\frac{dS_1}{dt} = -\frac{k_{max1}X}{Y_1}S_1 \tag{4.128}$$

$$y(t) = OUR_{ex}(t) = -(1-Y_1)\frac{dS_1}{dt} \tag{4.129}$$

The parameters for which we would like to check the structural identifiability are here Y_1, μ_{max1}, X, and $S_1(0)$ (since we have a priori no idea of the initial value of S_1 at the beginning of the respirometric experiment).

First note that considering the initial value of S_1 as a parameter is an extension of the cases considered in the preceding sections (yet already suggested by considering $x(0) = x_0(\theta)$ in equations (4.30), (4.41) and (4.46)). This case appears to be of great interest in several applications (like the one presented here).

The structural identifiability of the first model is rather straightforward.

Since the model is linear in the state variable $S_1(t)$ and of the output $OUR_{ex}(t)$, we can use the Laplace transform to perform the identifiability analysis. The Laplace transform $\mathcal{L}(s)$ applied to equations (4.128) and (4.129) gives:

$$\mathcal{L}(S_1) = \frac{S_1(0)}{s + \frac{k_{max1}X}{Y_1}} \tag{4.130}$$

$$\mathcal{L}(y) = \frac{(1-Y_1)k_{max1}X}{Y_1}\frac{S_1(0)}{s + \frac{k_{max1}X}{Y_1}} \tag{4.131}$$

We have a first order equation similar to the Laplace transform of equation (4.2). From the arguments developed in Section 4.3.2, we know that only two parameter combinations (corresponding to the numerator coefficient and to the denominator coefficient) will be identifiable. Indeed the inverse Laplace transform of (4.131) gives the following time evolution for $y(t)$:

$$y(t) = \frac{(1-Y_1)k_{max1}X}{Y_1}S_1(0)e^{-\frac{k_{max}Xt}{Y_1}} \tag{4.132}$$

The initial value $y(0)$ gives the amplitude $\frac{(1-Y_1)k_{max1}X}{Y_1}S_1(0)$, and the time response (decrease of 95 % after three time constants, i.e. at $t = \frac{3Y_1}{k_{max}X}$) gives the time constant $\tau = \frac{Y_1}{k_{max}X}$.

Therefore we see that only the two parameter combinations $\theta_1 = (1 - Y_1)S_1(0)$ and $\theta_2 = \frac{k_{max}X}{Y_1}$ are identifiable. Note that Y_1 is identifiable if $S_1(0)$ is known.

Note also that we could have equivalently written the model equations in a linear regression form by considering for instance the integral of $OUR_{ex}(t)$ as the output (see also [74]):

$$y'(t) = \int_O^t OUR_{ex}(\tau)d\tau \tag{4.133}$$

This means that:

$$\frac{dy'}{dt} = OUR_{ex}(t) \tag{4.134}$$

From equation (4.129), we know that the integral of OUR_{ex}, $y'(t)$, is equal to:

$$y'(t) = -(1 - Y_1)(S_1(t) - S_1(0)) \tag{4.135}$$

By combining equations (4.128) and (4.129), we can write $S_1(t)$ as a function of $OUR_{ex}(t)$:

$$S_1(t) = \frac{Y_1}{(1 - Y_1)k_{max1}X} OUR_{ex}(t) \tag{4.136}$$

By introducing (4.133), (4.134) and (4.136) into equation (4.135), we obtain:

$$\frac{dy'}{dt} = \beta_1 y' + \beta_2 \tag{4.137}$$

with:

$$\beta_1 = -\frac{k_{max1}X}{Y_1},\ \beta_2 = \frac{(1 - Y_1)k_{max1}X}{Y_1} S_1(0) \tag{4.138}$$

Observe that the two parameter combinations β_1 and β_2 are structurally identifiable from the data of OUR_{ex} and their time integral (4.133). Let us illustrate the structural identifiability concept via (real-life) data (Figure 4.2) with an initial substrate concentration $S_1(0)$. Figure 4.3 shows the data pairs (OUR_{ex}, $\int_o^t OUR_{ex}(\tau)d\tau$) corresponding to the ($OUR_{ex}(t)$, t) data presented in Figure 4.2: β_2 is given by the initial value of OUR_{ex}, and β_1 is the slope.

4.6.2 *Identifiability of the Single Monod Model (Taylor Series Expansion)*

Let us consider the identifiability properties of the second model (Single Monod with one substrate):

$$\frac{dS_1}{dt} = \frac{\mu_{max1}X}{Y_1} \frac{S_1}{K_{S1} + S_1} \tag{4.139}$$

Let us now use the Taylor series expansion method. This means that we look at the series expansion of $OUR_{ex}(t)$ around time t=0:

$$OUR_{ex}(t) = OUR_{ex}(0) + t\frac{dOUR_{ex}}{dt}(0) + \frac{t^2}{2!}\frac{d^2OUR_{ex}}{dt^2}(0) + ... \tag{4.140}$$

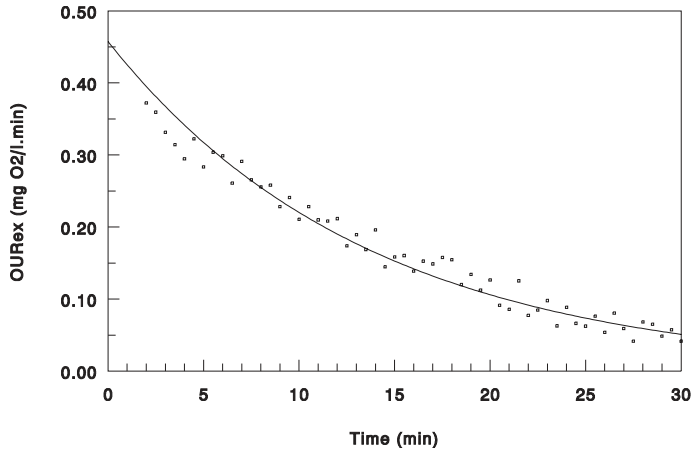

FIG. 4.2. OUR data corresponding to the exponential model.

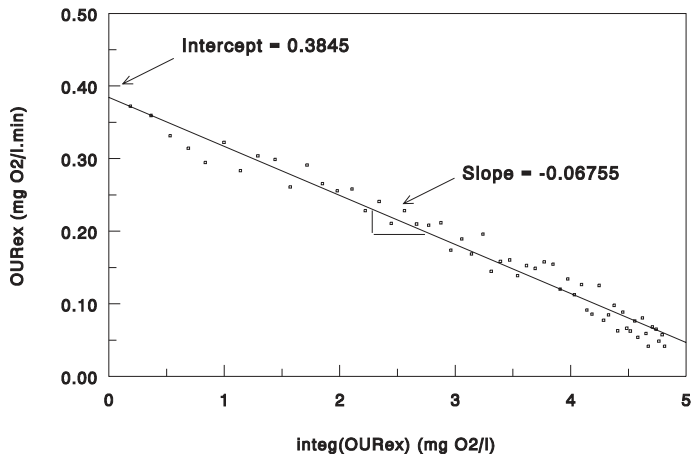

FIG. 4.3. Transformation of the OUR data into a linear regression form.

Let us compute the successive derivatives of $OUR_{ex}(t)$ at t = 0. The first terms will be written as follows:

$$OUR_{ex}(0) = \frac{\mu_{max1} X (1 - Y_1)}{Y_1} \frac{S_1(0)}{K_{S1} + S_1(0)} \tag{4.141}$$

$$\frac{dOUR_{ex}}{dt}(0) = -\frac{\mu_{max1}^2 X^2 (1 - Y_1)}{Y_1^2} \frac{K_{S1} S_1(0)}{(K_{S1} + S_1(0))^3} \tag{4.142}$$

$$\frac{d^2 OUR_{ex}}{dt^2}(0) = \frac{\mu_{max1}^3 X^3 (1-Y_1)}{Y_1^3} \frac{K_{S1} S_1(0)(K_{S1} - 2S_1(0))}{(K_{S1} + S_1(0))^5} \tag{4.143}$$

$$\frac{d^3 OUR_{ex}}{dt^3}(0) = -\frac{\mu_{max1}^4 X^4 (1-Y_1)}{Y_1^4} \frac{K_{S1} S_1(0)}{(K_{S1} + S_1(0))^7} (K_{S1}^2 - 8K_{S1} S_1(0) + 6S_1(0)^2) \tag{4.144}$$

$$\frac{d^4 OUR_{ex}}{dt^4}(0) = \frac{\mu_{max1}^5 X^5 (1-Y_1)}{Y_1^5} \frac{K_{S1} S_1(0)}{(K_{S1} + S_1(0))^9} (K_{S1}^3 - 22K_{S1}^2 S_1(0) + 58K_{S1} S_1(0)^2 - 24S_1(0)^3) \tag{4.145}$$

The number of parameters has now increased: there are five parameters to be identified: Y_1, μ_{max1}, X, K_{S1} and $S_1(0)$.

The key question is then the following: are they all structurally identifiable, or only combinations of them?

Let us first note that the following parameter combinations:

$$\theta_1 = \frac{\mu_{max1} X (1-Y_1)}{Y_1},\ \theta_2 = (1-Y_1) S_1(0),\ \theta_3 = (1-Y_1) K_{S1} \tag{4.146}$$

are combined in the first three derivatives. Indeed by noting:

$$z_i = \frac{d^i OUR_{ex}}{dt^i}(0),\ i = 0,\ 1,\ 2, ... \tag{4.147}$$

equations (4.141), (4.142) and (4.143) can be rewritten under the following (equivalent) form:

$$z_0 = \frac{\theta_1 \theta_2}{\theta_2 + \theta_3} \tag{4.148}$$

$$z_1 = -\frac{\theta_1^2 \theta_2 \theta_3}{(\theta_2 + \theta_3)^3} \tag{4.149}$$

$$z_2 = \frac{\theta_1^3 \theta_2 \theta_3 (\theta_3 - 2\theta_2)}{(\theta_2 + \theta_3)^5} \tag{4.150}$$

Therefore the "parameters" θ_1, θ_2 and θ_3 can be formally calculated from the values of z_i (which can be theoretically calculated from a (OUR(t), t) dataset) by inverting the above expressions, i.e.:

$$\theta_1 = \frac{z_0 (z_0 z_2 - 3z_1^2)}{z_0 z_2 - z_1^2} \tag{4.151}$$

$$\theta_2 = -\frac{2z_0^2 z_1}{z_0 z_2 - 3z_1^2} \tag{4.152}$$

$$\theta_3 = -\frac{4z_0^2 z_1^3}{(z_0 z_2 - 3z_1^2)(z_0 z_2 - z_1^2)} \tag{4.153}$$

The question is then the following: can we expect to increase the number of identifiable parameters by considering higher order derivatives?

If we look at the additional derivatives for i $\geq$ 3 (e.g. 4.145), the above parameter combinations are still combined basically in the same way as for the lower derivative terms, without any possibility to put in evidence other parameter combinations which could lead to a larger set of identifiable parameters. The conclusion appears to remain the same (we can never be sure!) if we consider even higher order derivatives: only the above parameter combinations θ_1, θ_2 and θ_3 are structurally identifiable.

The above set of parameter combinations is not the only one that fits in the above identifiability analysis. Other combinations can also be considered (e.g. θ_1 and θ_2 as in (4.146), and $\theta_3 = (1 - Y_1)(K_{S1} + S_1(0))$, but they are combinations of the above parameter combinations (4.146), and therefore result basically in the same conclusions to the one given above. Note also that the method using the transformation of the nonlinear model has also been applied to the Single Monod model and leads to the same conclusions.

Finally it is worth noting that symbolic manipulation software has been used to compute the successive derivatives and, once a set of parameter combinations was chosen, to perform the subsequent computations (e.g. (4.148), (4.149), (4.150) and (4.151), (4.152), (4.153) above).

4.6.3 *Identifiability of the Double Monod Model (Nonlinear Transformation)*

Let us now consider the Double Monod model (two pollutants simultaneously degraded without mutual interaction, (k=2)):

$$\frac{dS_1}{dt} = -\frac{\mu_{max1} X}{Y_1} \frac{S_1}{K_{S1} + S_1} \tag{4.154}$$

$$\frac{dS_2}{dt} = -\frac{\mu_{max2} X}{Y_2} \frac{S_2}{K_{S2} + S_2} \tag{4.155}$$

Let us here find a transformation of the nonlinear model into a model linear in the parameters. The line of reasoning is basically similar to the one considered for the exponential model above (development (4.134)-(4.138)). Recall that here the oxygen uptake rate OUR_{ex} is the sum of the contribution of two substrates S_1 and S_2:

$$OUR_{ex} = -(1 - Y_1) r_{S_1} - (1 - Y_2) r_{S_2} \tag{4.156}$$

A typical OUR_{ex} profile is shown in Figure 4.4. In the following, we assume (as it is suggested in Figure 4.4) that one substrate (S_1) is completely eliminated from the mixed liquor after the first part of the experiment (note that there is only one pathological case when this assumption does not hold: when S_1 and S_2 are

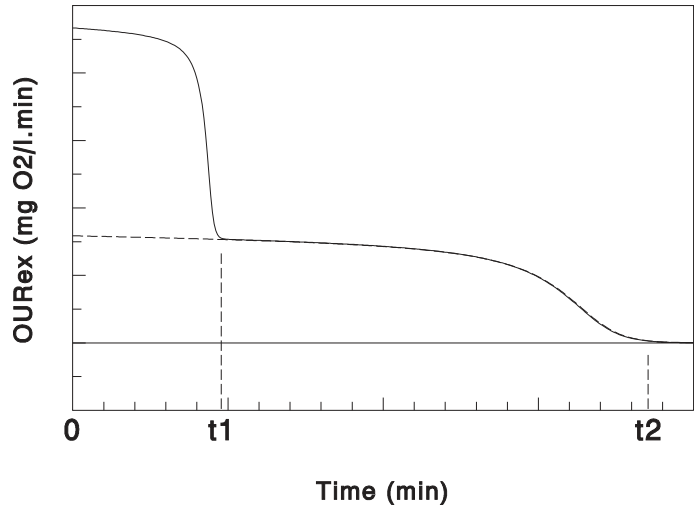

FIG. 4.4. Conceptual OUR profile with double Monod kinetics.

eliminated at exactly the same time). With this assumption the oxygen uptake rate can be subdivided in two parts corresponding with the degradation of each substrate. Hence, the identifiability analysis reduces to the analysis of the Single Monod model performed in two steps: for $0 \leq t < t_1$ for the first Monod model, and for $t_1 \leq t \leq t_2$ for the second one.

Let us insist that the present procedure is only valid in the presence of fully decoupled degradation of the substrates S_1 and S_2.

Let us first proceed for the first step and denote the first term of the right hand side of (4.156) by OUR_1:

$$OUR_1 = -(1 - Y_1)\frac{dS_1}{dt} \tag{4.157}$$

The integration of the above equation gives:

$$S_1(t) = S_1(0) - \frac{1}{1 - Y_1}\int_0^t OUR_1(\tau)d\tau \tag{4.158}$$

By introducing equation (4.154), the oxygen uptake rate equation (4.157) can be rewritten as follows:

$$OUR_1 = \frac{1 - Y_1}{Y_1}\frac{\mu_{max1}S_1}{K_{S1} + S_1}X \tag{4.159}$$

By multiplying both sides of the above equation by $(K_{S1} + S_1)$, and by considering (in order to have more compact notations) the variable $y_1(t)$ defined as follows:

$$y_1(t) = \int_0^t OUR_1(\tau)d\tau \tag{4.160}$$

equation (4.159) after some manipulations becomes:

$$y_1 \frac{dy_1}{dt} = -\alpha_1 - \alpha_2 y_1 + \alpha_3 \frac{dy_1}{dt} \tag{4.161}$$

where the parameters α_i (i = 1, 2, 3) are indeed combinations of the parameters θ_i (i = 1, 2, 3) defined in (4.146):

$$\alpha_1 = \theta_1\theta_2,\ \alpha_2 = \theta_1,\ \alpha_3 = \theta_2 + \theta_3 \tag{4.162}$$

There is clearly a one-to-one relation between these two sets of parameters:

$$\theta_1 = \alpha_2,\ \theta_2 = \frac{\alpha_1}{\alpha_2},\ \theta_3 = \alpha_3 - \frac{\alpha_1}{\alpha_2} \tag{4.163}$$

Then we can conclude that with independent data of $y_1 \frac{dy_1}{dt}$, y_1 and $\frac{dy_1}{dt}$ (generated via an appropriate experiment design), the parameters $\alpha_1, \alpha_2, \alpha_3$, and therefore the parameters $\theta_1, \theta_2, \theta_3$, are identifiable.

This result corresponds to the one obtained in the preceding section for the Single Monod model (for which we used the Taylor series expansion approach).

We can proceed similarly for the second step $t_1 \leq t \leq t_2$, and using similar definitions for OUR_2 and $y_2(t)$, it is straightforward that the parameters:

$$\theta_4 = \frac{\mu_{max2} X(1 - Y_2)}{Y_2},\ \theta_5 = (1 - Y_2)S_2(0),\ \theta_6 = (1 - Y_2)K_{S2} \tag{4.164}$$

are identifiable. This means that only six combinations of the nine original parameters (Y_1, $S_1(0)$, μ_{max1}, K_{S1}, Y_2, $S_2(0)$, μ_{max2}, K_{S2}, and X) are structurally identifiable.

4.6.4 *Identifiability of a Modified ASM1 Model (Nonlinear Transformation and Generating Series)*

The analysis carried out for the modified ASM1 model is based on the following model equations (3 pollutants, 2 hydrolysed into the first substrate which is used for growth according to the Monod kinetics):

$$\frac{dS_1}{dt} = -\frac{\mu_{max1} X}{Y_1} \frac{S_1}{K_{S1} + S_1} + k_r X_r + k_s X_s \tag{4.165}$$

$$\frac{dX_r}{dt} = -k_r X_r \tag{4.166}$$

$$\frac{dX_s}{dt} = -k_s X_s \tag{4.167}$$

and the oxygen uptake rate is written as follows:

$$OUR_{ex} = \frac{\mu_{max1} X (1 - Y_1)}{Y_1} \frac{S_1}{K_{S1} + S_1} \tag{4.168}$$

As in Section 4.6.3, the first step in the analysis consists of considering that during a part of the experiment, the concentration of the rapidly hydrolysable substrate X_r should be approximately zero.

The effects of the two substrates cannot be decoupled, unlike in the Double Monod model where this was possible due to the saturation in the kinetics of S_2. Yet we can assume that the rapidly biodegradable substrate X_r is completely consumed after a time $t = t_1$. Therefore we can perform the analysis in two steps.

1. Step 1: $t_1 \leq t \leq t_2$ when X_r is assumed to be equal to zero. This means that in the first step only equations (4.165), (4.167) and (4.168) with $X_r = 0$ are considered.
2. Step 2: $0 \leq t < t_1$, with all equations (4.165), (4.166), (4.167) and (4.168) and the knowledge given.

Let us consider here two approaches: the nonlinear transformation, and the generating series.

Nonlinear transformation. The analysis is performed as follows:

1. Integration of equations (4.167) and (4.168). This gives the following relation for $S_1(t)$:

$$S_1(t) = S_1(0) - \tfrac{1}{1-Y_1} \int_0^t OUR_{ex}(\tau) d\tau + \int_0^t k_s X_s(\tau) d\tau \tag{4.169}$$

$$= S_1(0) - \tfrac{1}{1-Y_1} \int_0^t OUR_{ex}(\tau) d\tau + k_s X_s(0)(1 - e^{-k_s t}) \tag{4.170}$$

2. Linearisation of the exponential term $e^{-k_s t}$ around t = 0 (in order to carry out the analysis with a model linear in the parameters):

$$e^{-k_s t} \cong 1 - k_s t + \frac{k_s^2 t^2}{2} \tag{4.171}$$

(We stop the series expansion at the second order term since the additional terms do not add extra useful information for the analysis.)

3. Introduction of these results in equation (4.168) and rewriting by multiplying both sides by $(1 - Y_1)K_{m1} + (1 - Y_1)S_1(0) - \int_0^t OUR_{ex}(\tau) d\tau +$

$(1 - Y_1)k_s X_s(0)(k_s t - \frac{k_s^2 t^2}{2})$ and considering $y(t)$ defined as the integral of $OUR_{ex}(t)$, as in (4.133):

$$y\frac{dy}{dt} = \beta_1 + \beta_2 y + \beta_3 \frac{dy}{dt} + \beta_4 t\frac{dy}{dt} - \beta_5 \frac{t^2}{2}\frac{dy}{dt} - \beta_6 t + \beta_7 \frac{t^2}{2} \quad (4.172)$$

$$\beta_1 = -\theta_1\theta_2,\ \beta_2 = \theta_1,\ \beta_3 = \theta_2 + \theta_3 \quad (4.173)$$

$$\beta_4 = \theta_7\theta_8^2,\ \beta_5 = \theta_7\theta_8^3,\ \beta_6 = \theta_1\beta_4,\ \beta_7 = \theta_1\beta_5 \quad (4.174)$$

with θ_1, θ_2, θ_3 as defined in (4.146), and θ_7 and θ_8 defined as follows:

$$\theta_7 = (1 - Y_1)X_s(0),\ \theta_8 = k_s \quad (4.175)$$

Among the seven parameters β_i, only five are independent (β_6 and β_7 are related to β_4, β_5 and β_2). Therefore five parameter combinations are identifiable, i.e. β_1, β_2, β_3, β_4 and β_5, or equivalently θ_1, θ_2, θ_3, θ_7 and θ_8.

The second step for $0 \leq t \leq t_1$ considers that the dynamics are given by equations (4.165), (4.166), (4.167) and (4.168) with $X_r \neq 0$, and that the values of the parameters β_i (i = 1 to 7) are already available from the first step, i.e. from data for times between t_1 and t_2. Then by following the same line of reasoning as in step 1, one obtains two more identifiable parameter combinations: $(1 - Y_1)X_r(0)$ and k_r.

Generating series. The equations (4.41) and (4.42) specialise here as follows:

$$x = \begin{pmatrix} S_1 \\ X_s \end{pmatrix},\ f_0 = \begin{pmatrix} -\frac{\mu_{max1}X}{Y_1}\frac{S_1}{K_{S1}+S_1} + k_s X_s \\ -k_s X_s \end{pmatrix} \quad (4.176)$$

$$h(x,\theta) = (1 - Y_1)\frac{\mu_{max1}X}{Y_1}\frac{S_1}{K_{S1} + S_1} - (1 - Y_1)k_s X_s \quad (4.177)$$

$$x(0) = x_0(\theta) = \begin{pmatrix} S_1(0) \\ X_s(0) \end{pmatrix} \quad (4.178)$$

From the knowledge that we have gained from the preceding examples, we can suspect that the individual parameters μ_{max1}, X and Y_1 will not be identifiable, but only the combination $\frac{\mu_{max1}X}{Y_1}$. For simplifying the rest of the developments, let us consider it as one parameter. Therefore we can see that we have six parameters:

$$\theta_1 = \frac{\mu_{max1}X}{Y_1} \quad (4.179)$$

$$\theta_2 = (1 - Y_1) \quad (4.180)$$

$$\theta_3 = K_{S1} \quad (4.181)$$

$$\theta_4 = k_s \quad (4.182)$$

$$\theta_5 = S_1(0) \tag{4.183}$$
$$\theta_6 = X_s(0) \tag{4.184}$$

Let us now calculate $h(0, \theta)$ and the successive Lie derivatives.

Since the calculations are quite complex and the resulting equations are very much involved, we shall concentrate on the first four elements calculated in the first step. (Let us recall that at least six elements are necessary to complete the analysis.) Let us first define these first four terms as follows:

$$h(0, \theta) = z_1 \tag{4.185}$$
$$L_{f_0} h(0, \theta) = z_2 \tag{4.186}$$
$$L_{f_0} L_{f_0} h(0, \theta) = z_3 \tag{4.187}$$
$$L_{f_0} L_{f_0} L_{f_0} h(0, \theta) = z_4 \tag{4.188}$$

Then the calculations give:

$$z_1 = \theta_2\theta_1 \frac{\theta_5}{\theta_3 + \theta_5} - \theta_2\theta_4\theta_6 \tag{4.189}$$
$$z_2 = -\frac{\theta_1\theta_3}{(\theta_3 + \theta_5)^2} z_1 + \theta_2\theta_4^2\theta_6 \tag{4.190}$$
$$z_3 = -\frac{\theta_1\theta_3}{\theta_2(\theta_3 + \theta_5)^3} z_1^2 - \frac{\theta_1\theta_3}{(\theta_3 + \theta_5)^2} z_2 - \theta_2\theta_4^3\theta_6 \tag{4.191}$$
$$\begin{aligned} z_4 = &-\frac{6\theta_1\theta_3}{\theta_2^2(\theta_3 + \theta_5)^4} z_1^3 + \frac{6\theta_1^2\theta_3^2}{\theta_2(\theta_3 + \theta_5)^5} z_1^2 \\ &-\frac{\theta_1^3\theta_3^3}{(\theta_3 + \theta_5)^6} z_1 - \frac{3\theta_1\theta_3}{\theta_2(\theta_3 + \theta_5)^3} z_1 z_2 \\ &-4\theta_4^2 \frac{\theta_1\theta_3\theta_6}{(\theta_3 + \theta_5)^3} z_1 - \theta_4^2 \frac{\theta_1\theta_2\theta_3\theta_6}{(\theta_3 + \theta_5)^2} \left(\frac{\theta_1\theta_3}{(\theta_3 + \theta_5)^2} - \theta_4\right) + \theta_2\theta_4^4\theta_6 \end{aligned} \tag{4.192}$$

Although we do not have enough information from the above equations to conclude, these already contain useful hints about the identifiability.

- First note that the last term of the right hand side of each equation (4.189), (4.190), (4.191) and (4.192) is a combination of three parameters (θ_2, θ_4 and θ_6). Among the three, only θ_4 appears with an increasing power. This suggests that θ_4 (= k_s) might be identifiable (*hint #1*). For the other two parameters, we have to investigate further.
- Let us now look at z_1 (4.189). We note that θ_2 appears linearly in both terms. In other words, the parameters θ_1, θ_2 and θ_6 appear to be linked in the following combinations $\theta_2\theta_1$ and $\theta_2\theta_6$ (*hint #2*).
- For z_2 (4.190), we note that the term multiplying z_1 is a fraction where the degree of the numerator with respect to the parameters is equal to the degree

of the denominator (numerator: degree 1 for θ_1, degree 1 for θ_3; denominator: degree 2 for $\theta_3 + \theta_5$).

- Let us look at all the terms but the last one (already considered here above) in each equation (4.189), (4.190), (4.191) and (4.192). We note that θ_2 appears whenever the degree of the numerator is different from the degree of the denominator. More precisely, it appears with a power equal to the difference between both degrees. (Equivalently each term can be rewritten with θ_2 appearing both at the numerator and the denominator with a degree equal to the total degree of the parameters of the numerator and the denominator, respectively.) This suggests that the four parameters θ_1, θ_3, θ_5 and θ_6 cannot be separated from θ_2, or in other words, that only the combinations $\theta_1\theta_2$, $\theta_2\theta_3$, $\theta_2\theta_5$, $\theta_2\theta_6$ (*hint #3*) can.

From the above comments, we have hints of what we may expect in terms of structural identifiability. These are in line with the results that we had obtained with the preceding examples (first order kinetics, single Monod, double Monod). Let us insist that this preliminary analysis does not allow us to make a conclusion. Only a complete analysis (with at least two extra generating series terms) will allow us to give results. This full analysis has indeed been performed. It shows that five parameter combinations

$$\theta_4 = k_s \tag{4.193}$$

$$\theta_1\theta_2 = \frac{\mu_{max1} X (1 - Y_1)}{Y_1} \tag{4.194}$$

$$\theta_2\theta_3 = (1 - Y_1) K_{S1} \tag{4.195}$$

$$\theta_2\theta_5 = (1 - Y_1) S_1(0) \tag{4.196}$$

$$\theta_2\theta_6 = (1 - Y_1) X_s(0) \tag{4.197}$$

are identifiable in step 1 (since we can write them as functions of the z_i). This confirms the results obtained with the nonlinear transformation.

4.6.5 *Summary of the Results and Discussion*

The identifiability results for the four models are summarised in Table 4.1.

The examples presented in Sections 4.6.1, 4.6.2 and 4.6.3 are illustrative of the advantages and drawbacks of the considered methods. The implementation of the series expansion method has the advantage of being systematic in the sense that it follows a clearly identified route. The above example is illustrative of the potential difficulties with the series expansion:

- How many derivatives of OUR_{ex} are needed to obtain conclusive results? For certain models the question may rise whether one can achieve better identifiability properties by considering more terms in the expansion. Here

Table 4.1 Identifiable parameter combinations of the four models

Exponential	Single Monod	Double Monod	Modified ASM1
$(1-Y_1)S_1(0)$	$(1-Y_1)S_1(0)$	$(1-Y_1)S_1(0)$	$(1-Y_1)S_1(0)$
$\frac{k_{max1}X}{Y_1}$	$\frac{\mu_{max1}X(1-Y_1)}{Y_1}$	$\frac{\mu_{max1}X(1-Y_1)}{Y_1}$	$\frac{\mu_{max1}X(1-Y_1)}{Y_1}$
	$(1-Y_1)K_{S1}$	$(1-Y_1)K_{S1}$	$(1-Y_1)K_{S1}$
		$(1-Y_2)S_2(0)$	$(1-Y_1)X_r(0)$
		$\frac{\mu_{max2}X(1-Y_2)}{Y_2}$	k_r
		$(1-Y_2)K_{S2}$	$(1-Y_1)X_s(0)$
			k_s

we found that the additionally evaluated terms did not yield additional information. Generally speaking the approach may imply more and more symbolic computations, and yet not lead to conclusive results (as experienced for the Double Monod model).

- How can we derive the right combinations of identifiable parameters? There is indeed no general systematic rule for selecting these combinations, and therefore the procedure may look a little tricky. However the structure of the different terms of the expansion is often a source for good initial guesses. For instance, the choice of θ_1 $(= -\frac{\mu_{max1}X(1-Y_1)}{Y_1})$ looks quite obvious from equations (4.141), (4.142) and (4.143).

On the other hand, the nonlinear transformation may suffer from the difficulty to easily find out the transformation that will a priori suit the problem (although in the proposed example, the choice of the transformation (multiplication by the denominator of the Monod model) is rather straightforward).

Let us also make some comments about the obtained identifiability results.

First note that the yield coefficient(s) Y_1 (Y_2) is present in all the parameter combinations (except in k_r and k_s in the modified ASM1 model). This is not surprising since, on the basis of OUR_{ex} data only, one can have no idea which quantity of substrate has been transformed into biomass. This explains why the term $(1-Y_1)S_1(0)$ $((1-Y_2)S_2(0))$, i.e. the fraction of substrate which is oxidised, appears as a parameter combination. The same remark applies to $(1-Y_1)K_{S1}$ $((1-Y_2)K_{S2})$ which can be viewed as no more than a rescaling of the substrate affinity constant.

Secondly the parameter combination $\frac{\mu_{max1}X(1-Y_1)}{Y_1}$ $(\frac{\mu_{max2}X(1-Y_2)}{Y_2})$ is an expression of the total activity of the sludge, and with that respect can be considered as giving information different from that of the individual parameters.

Finally let us point out that *a priori* information about some individual parameters (e.g. the yield coefficient(s) Y_1 (Y_2) values obtained via separate experi-

ments) can be incorporated in the parameter evaluation procedure. Then individual parameters (e.g. $S_1(0)$ or K_{S1}) can be estimated. As a matter of example, in the single Monod model, if $S_1(0)$ is known, then Y_1 is identifiable, and consequently, K_{S1} is also identifiable.

4.7 General Structural Identifiability Results for the ASM-type Models

Petersen *et al*. [194] propose a generalisation of the structural identifiability results for the ASM1. The proposed generalisation applies to models like the ASM1 where the kinetics are either first order kinetics or Monod-type models. It is based on measured variables that are either concentrations of components appearing explicitly in the mass balance models or directly related to these (like the OUR, which has been linked to the substrate concentration in the respirometer-based examples of the preceding section). Finally the results apply to one reaction at a time: in that sense, they apply to the ASM1 under the assumption that each reaction can be decoupled from the others (an assumption that obviously has to be validated, see also the preceding section for examples).

The generalisation results are summarised in Table 4.2, for two cases: when one measurement is available and when two measurements are available. They are based on the tabular form of the ASM1 (Section 2.3.3). The terms ν correspond to the yield coefficients, the terms K correspond to the saturation constant in the kinetic Monod models, the indices i and j correspond to the number of the measured component (column) in the ASM1 table, and the number of the process (reaction) (row) in the same table. More precisely, j corresponds to one or two columns in the ASM1 table (depending on the number of measured components), and i corresponds to the rows in the ASM1 tables, corresponding to the processes (reactions) considered in the dynamical model.

It is important to note that so far there is no technical (or mathematical) proof that the proposed results represent a generalisation. On the other hand, all the examples that have been treated so far give identifiablity results that follow the proposed generalisation.

4.8 Overparametrisation: An Illustrative Example

So far, we have introduced different methods for testing the structural identifiability and illustrated them on several examples. Typically, when the number of identifiable parameters is lower than the original number of parameters, the model is said to be overparametrised. Overparametrisation can indeed be detected in many instances via a preliminary analysis based for instance on a transformation of the original model formulation into an equivalent one in which the number of parameters may be lower. Formally this means that, if we consider the following system dynamics:

Table 4.2 Identifiable parameter combinations of the ASM-type model: generalisation for one and two measurements

One measurement (j)		*Two measurements (j)*	
No growth	Growth	No growth	Growth
$\mid \nu_{i,j} \mid \mu_{max,j} X$	$\mu_{max,j}$	$\mid \nu_{i,j} \mid \mu_{max,j} X$	$\mu_{max,j}$
$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid K_j$	$\mid \nu_{i,j} \mid X(0)$	$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid K_j$	$\mid \nu_{i,j} \mid X(0)$
$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid S_k(0)$	$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid K_j$	$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid S_k(0)$	$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid K_j$
	$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid S_k(0)$		$\mid \frac{\nu_{i,j}}{\nu_{k,j}} \mid S_k(0)$
		$\mid \frac{\nu_{i(1),j}}{\nu_{i(2),j}} \mid$	$\mid \frac{\nu_{i(1),j}}{\nu_{i(2),j}} \mid$

$$\frac{dx}{dt} = f(\theta, x, u) \tag{4.198}$$

$$y = h(\theta, x) \tag{4.199}$$

and if we find a state transformation:

$$x' = g(x) \tag{4.200}$$

such that the new model formulation:

$$\frac{dx'}{dt} = f'(\theta', x', u) \tag{4.201}$$

$$y = h'(\theta', x') \tag{4.202}$$

contains less parameters:

$$dim(\theta') < dim(\theta) \tag{4.203}$$

then the original model is overparametrised, and only the parameters θ' can possibly be structurally identifiable.

Let us illustrate this with an example. Let us consider the following dynamical model of an anaerobic digestion process ([15]):

$$\frac{dS_0}{dt} = -DS_0 + \alpha DS_{in} - k_0 S_0 X_1 \tag{4.204}$$

$$\frac{dS_1}{dt} = -DS_1 + k_0 S_0 X_1 - \frac{1}{Y_1}\frac{\mu_{max1} S_1}{K_{S1} + S_1} X_1 \tag{4.205}$$

$$\frac{dX_1}{dt} = -DX_1 + \frac{\mu_{max1} S_1}{K_{S1} + S_1} X_1 - k_{d1} X_1 \tag{4.206}$$

$$\frac{dS_2}{dt} = -DS_2 + Y_3\frac{\mu_{max1} S_1}{K_{S1} + S_1} X_1 - \frac{1}{Y_2}\frac{\mu_{max2} S_2}{K_{S2} + S_2} X_2 \tag{4.207}$$

$$\frac{dX_2}{dt} = -DX_2 + \frac{\mu_{max2} S_2}{K_{S2} + S_2} X_2 - k_{d2} X_2 \tag{4.208}$$

$$Q_{CH_4} = Y_4\frac{\mu_{max2} S_2}{K_{S2} + S_2} X_2 \tag{4.209}$$

i.e. a model with three steps: solubilisation of organic compounds S_0 (equation (4.204)), acidification of solubilised substrate S_1 (equations (4.205)(4.206)), and methanisation of volatile fatty acids S_2 (4.207)(4.208)(4.209). Note that in this model compared to the one presented in Chapter 2, a solubilisation step has been added. X_1, X_2, S_{in} and Q_{CH_4} are the concentrations of acidogenic bacteria and of methanogenic bacteria, the concentration of nonsolubilised organic matter in the influent, and the methane gas outflow rate, respectively. Y_i (i=1 to 4) are yield coefficients, α is an availability coefficient, and k_0 is the kinetic constant of the solubilisation reaction. μ_{maxi}, K_{Si} and k_{di} (i=1, 2) are the maximum specific growth rates, the affinity constants, and the death coefficients related to acidogenesis and methanisation, respectively.

The above model contains 12 parameters (Y_1, Y_2, Y_3, Y_4, α, k_0, μ_{max1}, K_{S1}, k_{d1}, μ_{max2}, K_{S2}, k_{d2}). In the identifiability study performed in [15], the measured variables were the dilution rate D, the influent substrate concentration S_{in}, and the methane gas flow rate Q_{CH_4}. The dilution rate D and the influent substrate concentration S_{in} are typically actions (*inputs*) on the process, while the methane gas flow rate Q_{CH_4} is typically a result (*output*) of the process operation. This means that the output and input are defined as follows:

$$u = \begin{bmatrix} D \\ S_{in} \end{bmatrix}, \; y = Q_{CH_4} \tag{4.210}$$

Let us define the following state transformation:

$$\tilde{X}_1 = k_0 X_1 \tag{4.211}$$

$$\tilde{X}_2 = Y_4 X_2 \tag{4.212}$$

$$\tilde{S}_0 = \frac{S_0}{K_{S1}} \tag{4.213}$$

$$\tilde{S}_1 = \frac{S_1}{K_{S1}} \tag{4.214}$$

$$\tilde{S}_2 = \frac{S_2}{K_{S2}} \tag{4.215}$$

If we consider the following parameter combinations:

$$\tilde{Y}_1 = k_0 K_{S1} Y_1 \tag{4.216}$$

$$\tilde{Y}_2 = K_{S2} Y_4 Y_2 \tag{4.217}$$

$$\tilde{\alpha} = \frac{\alpha}{K_{S1}} \tag{4.218}$$

$$\tilde{Y}_3 = \frac{Y_3}{k_0 K_{S2}} \tag{4.219}$$

and by using the above state transformation (4.211)(4.215), the dynamical equations (4.204)-(4.209) can be represented by the following set of equations:

$$\frac{d\tilde{S}_0}{dt} = -D\tilde{S}_0 + \tilde{\alpha} D S_{in} - \tilde{S}_0 \tilde{X}_1 \tag{4.220}$$

$$\frac{d\tilde{S}_1}{dt} = -D\tilde{S}_1 + \tilde{S}_0 \tilde{X}_1 - \frac{1}{\tilde{Y}_1} \frac{\mu_{max1} \tilde{S}_1}{1 + \tilde{S}_1} \tilde{X}_1 \tag{4.221}$$

$$\frac{d\tilde{X}_1}{dt} = -D\tilde{X}_1 + \frac{\mu_{max1} \tilde{S}_1}{1 + \tilde{S}_1} \tilde{X}_1 - k_{d1} \tilde{X}_1 \tag{4.222}$$

$$\frac{d\tilde{S}_2}{dt} = -D\tilde{S}_2 + \tilde{Y}_3 \frac{\mu_{max1} \tilde{S}_1}{1 + \tilde{S}_1} \tilde{X}_1 - \frac{1}{\tilde{Y}_2} \frac{\mu_{max2} \tilde{S}_2}{1 + \tilde{S}_2} \tilde{X}_2 \tag{4.223}$$

$$\frac{d\tilde{X}_2}{dt} = -D\tilde{X}_2 + \frac{\mu_{max2} \tilde{S}_2}{1 + \tilde{S}_2} \tilde{X}_2 - k_{d2} \tilde{X}2 \tag{4.224}$$

$$Q_{CH_4} = \frac{\mu_{max2} \tilde{S}_2}{1 + \tilde{S}_2} \tilde{X}_2 \tag{4.225}$$

The above formulation is equivalent to the original one (4.204)-(4.209) with respect to the inputs (D, S_{in}) and the output Q_{CH_4}. The model contains now only eight parameters ($\tilde{Y}_1$, $\tilde{Y}_2$, $\tilde{Y}_3$, $\tilde{\alpha}$, μ_{max1}, k_{d1}, μ_{max2}, k_{d2}): this means that only these eight parameters are possibly structurally identifiable.

4.9 Conclusions

In this chapter, we have introduced the concept of structural identifiability. As stated in the introduction, the notion of structural identifiability may be essential

in the study of wastewater treatment processes and the use of dynamical models for numerical simulation, process design and/or control design, because the structural identifiability analysis will tell a priori if there is any chance that a candidate model is identifiable, i.e. if its parameters can be given unique values. This property is essential for the reliability of the model. If you have an unidentifiable model, this means that any numerical values of its parameters (as long as they correspond to a unique value of the identifiable parameter combinations[6]) can be given. How can you then have any confidence in such a model whether it is used for simulation, process design or control? And what kind of interpretation can you then give to the (physical) parameters of the model, if there exists an infinite possibility for choosing their values?

So far, we have introduced several tools to test the structural identifiability. These are:

1. Laplace transform,
2. Taylor series expansion,
3. generating series,
4. local state isomorphism,
5. transformation of nonlinear models,
6. Lyapunov-based observer analysis.

The first one was introduced in Section 6.3 and is only valid for linear models. However since most models in WWTP are nonlinear (not only in the state, but also in the parameter), it is important to propose tests that can be used to handle the structural identifiability of nonlinear models. These were introduced in Section 6.4 and 6.5. The method introduced in Section 6.5 (Lyapunov-based method) is quite a special one and difficult to generalise, especially for non-experts in system analysis and automatic control. Moreover, the motivation to introduce it was also a historical perspective with the first identifiability analysis of the Monod model. This motivated our choice to put it in a separate section. The methods introduced in Section 6.4 are indeed of a more general use, and are basically applicable to any model available in the literature on WWTP.

The use of these tools has been illustrated with several examples:

1. two interconnected tanks,
2. two reaction models,
3. Monod model,
4. respirometer-based models.

The basic features of these tools can be summarised as follows.

1. First of all, it should be noted that it is very difficult to a priori select the best method to test the structural identifiability of a dynamical model. It may happen that one method is much easier to apply to one model, and

[6] Assume that you have a model where $\theta_1 + \theta_2$ is identifiable (but not each parameter individually). It is obvious that any combination of values for θ_1 and θ_2 such that the sum is equal to a specific constant will give the same result in the model behaviour.

becomes completely cumbersome with another. This probably explains why there are (at least) six different structural identifiability methods available in the literature (see e.g. [55]).

2. Most of the structural identifiability tests only give local structural identifiability results for nonlinear models (except the local state isomorphism approach, and the Lyapunov approach if the domain of validity covers the whole physical space). The results given by these tests have been obtained for some specified time t (typically, t = 0) for the Taylor series expansion, or equivalently at the initial values for the generating series: therefore the results obtained are strictly speaking only valid for these values. In order to become "global", the approach should cover the whole physical state space via the computation of the different terms required for the test in this space for *all* admissible state variable values. For the transformation of nonlinear models, one should be particularly careful at some singularities (typically possible division by zero, for instance) that may arise during the transformation and back-transformation processes.
3. The methods may give sufficient or necessary identifiability results. The Taylor series expansion gives a *sufficient* [7] structural identifiability condition (see [197]) (because there exists no upper bound on the number of coefficients to be considered in the test). Generally speaking, the generating series method also gives sufficient conditions, but it results in necessary and sufficient conditions for bilinear and polynamial models.
4. The use of symbolic software can be very helpful to apply identifiability tests to the studied models. Very quickly the computation burden may become enormous, and without symbolic manipulation software, the computation may become impossible to handle in practice. The computation burden may be less important with the generating series approach than with the Taylor series approach. Yet this is not the panacea so far and symbolic software also exhibits its limitations: in several applications, it appears that the complexity of the required computations is such that the symbolic softwares that we have been using were not able to solve the problems (but of course, we can hope that this will improve in the future...).

Finally we have briefly introduced the notion of overparametrisation of dynamical models, and illustrated it with an anaerobic digestion model.

Different structural identifiability studies dedicated to water and wastewater treatment processes can be found in the literature.

[7] The notion of necessary and sufficient conditions is essential in mathematics. A condition C is sufficient means that if the condition C is fulfilled, the result R follows (in mathematical terms, C $\Rightarrow$ R). Yet this does not mean that if the condition C is not fulfilled, the result R is wrong or false. A necessary condition is the reciprocal proposal (R $\Rightarrow$ C): the result R is true or correct *only* if the condition C is fulfilled, and R may be false or wrong even if C is fulfilled.

We have presented here part of the results of the paper by Dochain *et al*. [74], illustrating the structural identifiability of different respirometer-based models was stabilised by considering various approaches including the Taylor series expansion and nonlinear transformations.

We have also mentioned the generalization effort done in Petersen [193] and Petersen *et al*. [194] for ASM1 type models (with first order kinetics and Monod kinetics) by considering respirometric measurements as well as combined respirometric-titrimetric measurements.

In Bourrel *et al*. [35], the authors analyse the identifiability of a denitrifying biofilter model. The process dynamics are described by partial differential equations (PDEs): this is one of the main original aspects of this work, especially with respect to what has been presented in the present chapter. There were seven measurement points along the column. The available data corresponds to different steady states: the authors have therefore studied the steady state equations (ordinary differential equations) for the identifiability analysis.

In Chen and Bastin [57], the authors consider the structural identifiability of the yield coefficients independently of the reaction rate model parameters. This analysis is made possible by considering the state transformation that we have considered in Chapter 2, and is based on the structural properties of the General Dynamical model.

The structural identifiability of the ASM model No. 1 and of a reduced-order version of this model is presented in Julien *et al*. (1992) [139], and Julien *et al*. (1998) [138], respectively. The model consists indeed in two submodels: one for aerobic conditions, the other one for anoxic conditions. The model was applied to an alternating operation of a WWTP. In the aerobic phase, the dynamical model is composed of three differential equations, while two differential equations describe the dynamics in anoxic conditions. The methods considered by the authors are local state isomorphism, and the transformation of the nonlinear model into a linear one (for the anoxic model in [138]).

In Keesman *et al*. [142], the authors study the identifiability of a model for endogenous respiration in an activated sludge in a batch reactor in the absence of dissolved oxygen limitation. This model is derived from the ASM1 model and contains six parameters (μ_m, Y, K_S, k_h, f_p, b). The structural identifiability is studied numerically by identifying the model parameters θ from a "thought-experiment" with a selected parameter vector θ^*, and by computing the gradient and the Hessian of the output prediction error in order to check that the solution $\theta = \theta^*$ is a local minimum. The authors obtain the following results. If only the endogenous respiration rate is measured, then k_h, f_p, and combinations of μ_m and K_S, and of Y, K_S and b (i.e. four parameters) are identifiable. If, in addition, measurements of the volatile suspended solids in the mixed liquor are available, then five parameters are identifiable: Y, k_h, f_p, b, and a combination of μ_m and K_S.

5

Practical Identifiability and Optimal Experiment Design for Parameter Estimation (OED/PE)

5.1 Introduction

In the preceding chapter, we discussed the notion of structural identifiability, which is related to the possibility of giving a unique value to each parameter of a mathematical model. The question that we addressed was the following: given a model structure and perfect (i.e. that fits perfectly to the model) data of model variables, are all the parameters of the model identifiable? A structural identifiablity study may result in the following conclusions. First it is possible that only combinations of the model parameters are identifiable. Moreover, if the number of resulting combinations is lower than the number of original model parameters, or if there is not a one-to-one relationship between both parameter sets, then a priori knowledge about some parameters may be required to achieve identifiability of each individual parameter.

In this chapter, we would like to discuss the notion of practical identifiability, which is the important complement to the structural identifiability in order to guarantee reliability of the calibration of the model parameters from available experimental data. Practical identifiability is indeed related to the quality of the

data and their "information" content: are the available data informative enough for identifying the model parameters and, more specifically, for giving accurate values? For instance in the model $y = ax_1 + bx_2$ the parameters a and b are structurally identifiable but they will not be practically identifiable if the experimental conditions are such that the independent variables x_1 and x_2 are always proportional ($x_1 = \alpha x_2$) (then only the *combination* $a\alpha + b$ is practically identifiable).

While the structural identifiability is studied under the assumption of perfect, i.e. noiseless, data, the problem with highly correlated parameters arises when a limited set of experimental, noise-corrupted data is used for parameter estimation. Under such conditions the uniqueness of parameter estimates predicted by the structural analysis may no longer be guaranteed because a change in one parameter can be compensated almost completely by a proportional shift in another, still producing a satisfying fit between experimental data and model predictions. In addition, the numerical algorithms that perform the nonlinear parameter estimation (presented in Chapter 6) show poor convergence when faced with this type of ill-conditioned optimisation problem, the estimates being very sensitive to the initial parameter values given to the algorithm [126], [171]. Consequently, the estimated parameters may vary over a broad range and little physical interpretation can be given to the parameter values obtained.

The Monod-model (μ_{max} is the maximum specific growth rate (min^{-1}), K_S is the saturation constant (mg/L)),

$$\mu(S) = \frac{\mu_{max} S}{K_S + S} \tag{5.1}$$

is probably the best-known example in biological systems of a model in which parameter estimates may be highly correlated [41],[126],[179]. In many cases the experiments provide only sufficient information to estimate the ratio between both parameters in this model, μ_{max}/K_S. A simple example may illustrate this (Figure 5.1): if only growth rates are available for low substrate concentrations (in the example of Figure 5.1, these range between 0 and 0.1 mg/L), no distinction can be made between different parameter sets, i.e. the Monod model is practically unidentifiable. In order to overcome this problem, it has been proposed to use additional a priori information (e.g. a known maximum growth rate), to impose parameter bounds [126], or to sample more frequently in defined periods of the experiment in order to increase the informative content of the collected data [276]. Evidently, measuring at higher substrate concentrations (see Figure 5.1, right) also allows unique, reliable parameter estimates to be obtained.

The chapter is organised as follows: we shall first discuss the concept of practical identifiability and the related notions of confidence intervals and sensitivity functions in Section 5.2. Section 5.3 will be devoted to optimal design of experiments in order to obtain the most reliable parameter values possible. The optimal experiment design will be illustrated in Section 5.4 with a respirometry-based

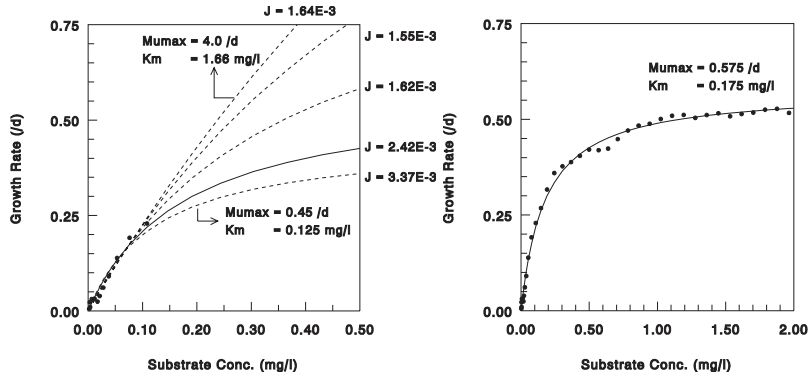

FIG. 5.1. Practical identifiability of the Monod model parameters (J represents the sum of the squared errors for different parameter sets).

model that we have already considered in Chapters 3 (Section 3.2) and 4 (Section 4.6). Finally the question of the optimal experiment design for the dual problem of structure characterisation and parameter estimation will be briefly discussed in Section 5.5.

5.2 Practical Identifiability

5.2.1 *Theoretical Framework*

The question addressed in this section is the following: with the available experimental data, what is the accuracy we can obtain for the parameter estimates, or, in other words, if a small deviation in the parameter set occurs, does this have a considerable decrease of the fit as a consequence. Mathematically, this can be formalized as follows [180].

Let us recall first (see also Chapter 6) that parameter estimation can often be formulated as the minimisation of the following quadratic objective functional by optimal choice of the parameters θ [180]:

$$J(\theta) = \sum_{i=1}^{N} (y_i(\hat{\theta}) - y_i)^T Q_i (y_i(\hat{\theta}) - y_i) \tag{5.2}$$

in which y_i and $y_i(\hat{\theta})$ are vectors of N measured values and model predictions at times t_i (i = 1 to N) respectively, and Q_i is a square matrix with user-supplied weighting coefficients. The expected value of the objective functional for a parameter set slightly different from the optimal one is given by [179]:

$$E[J(\theta + \delta\theta)] \cong \delta\theta^T [\sum_{i=1}^{N} (\frac{\partial y}{\partial \theta}(t_i))^T Q_i (\frac{\partial y}{\partial \theta}(t_i)] \delta\theta + \sum_{i=1}^{N} tr(C_i Q_i) \tag{5.3}$$

in which C_i represents the measurement error covariance matrix (Q_i is typically chosen as C_i^{-1} and the second term reduces to a scalar). An important consequence of (5.3) is that in order to *optimise the practical identifiability* one has to *maximise the term between brackets [.]* in equation (5.3). By doing so, one maximizes the difference between $J(\theta + \delta\theta)$ and $J(\theta)$ or in other words, one ensures that the fit of a parameter set that is slightly different from the best parameter set is significantly worse. The term between brackets in equation (5.3) is the so-called Fisher Information Matrix and expresses the information content of the experimental data [159]:

$$F = \sum_{i=1}^{N} (\frac{\partial y}{\partial \theta}(t_i))^T Q_i (\frac{\partial y}{\partial \theta}(t_i)) \tag{5.4}$$

This matrix is indeed the inverse of the parameter estimation error covariance matrix of the best linear unbiased estimator [99]:

$$V = F^{-1} = (\sum_{i=1}^{N} (\frac{\partial y}{\partial \theta}(t_i))^T Q_i (\frac{\partial y}{\partial \theta}(t_i)))^{-1} \tag{5.5}$$

The terms $\frac{\partial y}{\partial \theta}$ are the output sensitivity functions. These quantify the dependence of the model predictions on the parameter values. The evaluation of the sensitivity functions is a central task in the practical identifiability study and is dealt with in the next section.

As it will be discussed in detail in Chapter 6, the approximation (5.3) of the objective function allows one to draw lines of constant objective functional J values in the parameter space, and the delimited regions give confidence regions around the best parameter estimates for different confidence levels. In case a two-parameter problem is addressed, these lines form ellipses. As it is pointed out in Munack [179], the axes of the ellipses are given by the eigenvectors of the Fisher Matrix, and their lengths are proportional to the square root of the inverse of the corresponding eigenvalues. Hence, the ratio of the largest to the smallest (in absolute value) eigenvalue is a measure of the shape of the objective functional J close to the optimal parameter estimates.

It is important to note that many numerical optimization algorithms (needed to solve these non-linear parameter estimation problems) have difficulties in finding a global optimum in such valley-like functionals (for more details, see Chapter 6). The need to invert the Fisher Matrix in many of these algorithms is important in this respect [218]. Indeed, the above mentioned ratio of eigenvalues equals the Fisher Matrix's condition number which is a measure for the reliability by which the inversion can be made. Hence, if an appropriate experiment design could be found that alleviates this problem, increased estimation accuracy would result.

Petersen [193] studied this problem in more detail and concluded that these numerical problems can sometimes be solved by changing the units of the parameters

to be estimated. Indeed, the eigenvalues of the Fisher Matrix are unit-dependent. In case numerical problems are to be expected during parameter estimation, rescaling of the parameter units can be sufficient to alleviate these problems.

5.2.2 *Confidence Region of the Parameter Estimates*

A rather important result of a practical identifiability study is the possibility to determine the parameter estimation error. It can be stated that reporting parameter estimates without the corresponding parameter variance is meaningless as no confidence can be given to the parameter estimates. If the covariance matrix V (5.5) is available, and the matrix Q_i was defined as the inverse of the measurement error covariance matrix for calculation of the Fisher Matrix, approximate standard errors for the parameters can be calculated as:

$$\sigma(\theta_i) = \sqrt{V_{ii}} \tag{5.6}$$

Confidence intervals for the parameters are then obtained as:

$$\theta \pm t_{\alpha;N-p}\sigma(\theta_i) \tag{5.7}$$

for a confidence level specified as $100(1-\alpha)\%$ and t-values obtained from the Student-t distribution.

It should be mentioned though that these confidence intervals are too optimistic (too small) as they do not consider modelling errors. Indeed, only the measurement errors are included in the matrix Q_i.

In case only a single variable is measured, and fitted to, a more realistic estimate of the parameter confidence can be obtained by evaluating the residual mean square

$$s^2 = \frac{J_{opt}(\theta)}{N-p} \tag{5.8}$$

with p the number of parameters in the model and $J_{opt}(\theta)$ as defined in (5.2) and with Q_i a pxp identity matrix. Approximate standard errors for the parameters can then be calculated as:

$$\sigma(\theta_i) = s\sqrt{V_{ii}} \tag{5.9}$$

In this special case the standard errors are closer to the real ones since modelling errors are also included in $\sigma(\theta_i)$ since the J_{opt} contains both.

5.2.3 *Sensitivity Functions*

The output sensitivity $\partial y/\partial\theta$ equations are central to the evaluation of practical identifiability as they are a major component of the Fisher Information Matrix, and hence, also of the parameter estimation covariance matrix. If the sensitivity equations are proportional, the covariance matrix becomes singular and the model is not practically identifiable [218]. However, exceptions to this seem to exist. Petersen

et al. [195] reported that certain parameters were practically identifiable despite the fact that the sensitivity functions are proportional. It was argued that the non-linearity of the estimation problem was the reason for this. Evidently, it was not possible to calculate the parameter estimation error covariance matrix since inversion of the (singular) Fisher Matrix was impossible. However, other (exploratory) methods introduced in Chapter 6 allow the confidence region to be obtained.

Overall, however, for many models used to describe biological phenomena, the sensitivity equations are nearly proportional, resulting in parameter estimates that are highly correlated. This is also visualised in the error functional J that looks like a valley, i.e. several combinations of parameters may describe the same data (almost) equally well.

Therefore, an easy way to study the practical identifiability of a model is to plot the sensitivity equations. In the literature numerous studies can be found in which this study is performed, especially for the Single Monod model considering measurements of both biomass and substrate concentrations [126], [127], [170], [195], [201], [218], [276].

To obtain a particular sensitivity function $\frac{\partial y_j}{\partial \theta_i}$ different approaches are possible. The most accurate is the analytical derivation of the sensitivity function. For somewhat more complex models it quickly becomes necessary to use symbolic manipulation software to minimise the errors that would certainly slip into a manual derivation.

Alternatively a numerical approximation is possible. It basically requires additional evaluations of the model for parameter values that are slightly different from the nominal ones. Typically one parameter θ_i will be perturbed at a time with a properly chosen perturbation value $\Delta\theta_i$. The sensitivity of output y_j to θ_i is then easily calculated as

$$\frac{\partial y_j}{\partial \theta_i} = \frac{y_j(\theta_i) - y_j(\theta_i + \Delta\theta_i)}{\Delta\theta_i} \tag{5.10}$$

For illustrative purposes, the sensitivity equations are deduced for the Single Monod and modified IWA ASM model (see Chapter 3, Section 3.2) with OUR_{ex} measurements as the only source of information for the identification of the biokinetic parameters. The sensitivity of OUR_{ex} with respect to μ_{max1} is:

$$\frac{\partial OUR_{ex}}{\partial \mu_{max1}} = \frac{\partial}{\partial \mu_{max1}}\left(-(1 - Y_1)\frac{dS_1}{dt}\right) = -(1 - Y_1)\frac{d}{dt}\left(\frac{\partial S_1}{\partial \mu_{max1}}\right) \tag{5.11}$$

in which the state sensitivity $\frac{\partial S_1}{\partial \mu_{max1}}$ is obtained by integration of the differential equation (with zero initial value):

$$\frac{d}{dt}\left(\frac{\partial S_1}{\partial \mu_{max1}}\right) = \frac{\partial}{\partial \mu_{max1}}\left(-\frac{\mu_{max1} X}{Y_1}\frac{S_1}{K_{s1} + S_1}\right)$$

$$= -\frac{X}{Y_1}\left(\frac{S_1}{K_{s1}+S_1} + \frac{\mu_{max1}K_{s1}\frac{\partial S_1}{\partial \mu_{max1}}}{(K_{s1}+S_1)^2}\right) \tag{5.12}$$

where the substrate concentration S_1 is calculated by integration of the substrate dynamic model (note that X is assumed to be constant in the present model):

$$\frac{dS_1}{dt} = -\frac{\mu_{max1}X}{Y_1}\frac{S_1}{K_{s1}+S_1} \tag{5.13}$$

Simultaneous solution of the differential equations (5.12) and (5.13) allows the output sensitivities (5.11) to be calculated. One can proceed similarly for the sensitivity of OUR_{ex} with respect to K_{s1}. The following relations are obtained:

$$\frac{\partial OUR_{ex}}{\partial K_{s1}} = -(1-Y_1)\frac{d}{dt}\left(\frac{\partial S_1}{\partial K_{s1}}\right) \tag{5.14}$$

$$\frac{d}{dt}\left(\frac{\partial S_1}{\partial K_{s1}}\right) = -\frac{\mu_{max1}X}{Y_1}\left(\frac{K_{s1}\frac{\partial S_1}{\partial K_{s1}} - S_1}{(K_{s1}+S_1)^2}\right) \tag{5.15}$$

The equations show that the sensitivities of the Single Monod model are dependent on the parameter values. This is a general characteristic of nonlinear models that has even been used to define nonlinearity [80]. Consequently the Fisher Information Matrix (5.4) depends on the parameter values and this feature has important implications for the optimal experiment design (see below).

An example of an OUR_{ex} profile with the corresponding sensitivity function evolutions is given in Figure 5.2 (left). One observes that the sensitivity functions for K_{s1} and μ_{max1} are nearly proportional, a well-known characteristic of the Monod model. Intuitively, the sensitivity functions express the dependence of the output or state variable on a change in the parameters. Hence, the sensitivity functions indicate conditions where the dependence is the largest and therefore, under which conditions the most information can be gathered on the parameters. In the example of Figure 5.2 (left) these conditions prevail when the substrate concentration has dropped to a level close to the affinity constant K_{s1}. From this one can deduce a first approach to increase the information content of an experiment: choose the sampling times when the parameters are influent, i.e. in the high sensitivity zone [276].

The output sensitivities for the IWA ASM model as modified by Sollfrank and Gujer [233] are deduced in a similar manner (in case OUR_{ex} is the only measured variable and the biokinetic parameters μ_{max1}, K_{s1}, k_r and k_s are to be inferred):

$$\begin{aligned}\frac{\partial OUR_{ex}}{\partial k_r} &= (1-Y_1)\frac{\partial}{\partial k_r}\left(-\frac{dS_1}{dt} + k_r X_r + k_s X_s\right) \\ &= (1-Y_1)\left(X_r + k_r\frac{\partial X_r}{\partial k_r} - \frac{d}{dt}\left(\frac{\partial S_1}{\partial k_r}\right)\right)\end{aligned} \tag{5.16}$$

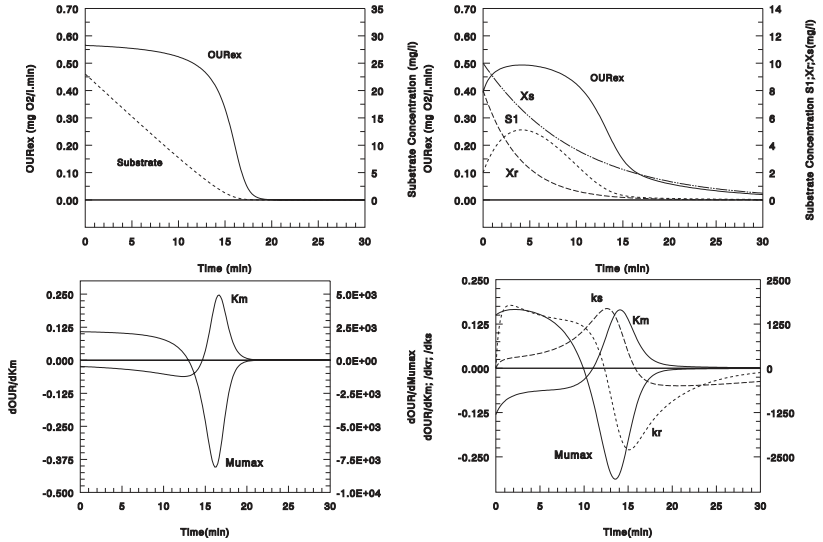

FIG. 5.2. Left: Output sensitivities (bottom) for a Single Monod-type OUR_{ex}-profile (top). Right: Output sensitivities (bottom) for an IWA ASM-type OUR_{ex}-profile (top).

$$\frac{\partial OUR_{ex}}{\partial k_s} = (1 - Y_1)\left(X_s + k_s \frac{\partial X_s}{\partial k_s} - \frac{d}{dt}\left(\frac{\partial S_1}{\partial k_s}\right)\right) \tag{5.17}$$

State sensitivities needed for the calculation of the output sensitivities (5.16)(5.17) are:

$$\frac{d}{dt}\left(\frac{\partial X_r}{\partial k_r}\right) = \frac{\partial}{\partial k_r}\left(-k_r X_r\right) = -\left(X_r + k_r \frac{\partial X_r}{\partial k_r}\right) \tag{5.18}$$

$$\frac{d}{dt}\left(\frac{\partial X_s}{\partial k_s}\right) = -\left(X_s + k_s \frac{\partial X_s}{\partial k_s}\right) \tag{5.19}$$

The output and state sensitivities for μ_{max1} and K_{s1} are identical to (5.11)(5.12) and (5.14)(5.15) respectively.

In Figure 5.2(right) the practical identifiability of the modified ASM model is studied by checking the output sensitivities for a short term batch experiment. No clear proportionality between sensitivity functions is observed. Stronger evidence can be obtained, however, by calculation of the rank of the Fisher Information Matrix. If no linear dependency exists, it should be full rank. This is indeed the case for this example. The condition number of the Fisher Matrix, or equivalently, the ratio of the largest to the smallest eigenvalue, indicates whether the sensitivities are nearly linearly dependent: the higher the condition number, the lower the practical identifiability.

So far, the initial conditions of the model variables were not included in the practical identifiability study, though they may be a highly desired outcome of parameter estimation. For the Single Monod model for instance, one can write:

$$\begin{aligned}\frac{\partial OUR_{ex}}{\partial S_1(0)} &= -(1-Y_1)\frac{d}{dt}\left(\frac{\partial S_1}{\partial S_1(0)}\right) \\ &= (1-Y_1)\frac{\mu_{max1}X}{Y_1}\frac{\partial}{\partial S_1(0)}\left(\frac{S_1}{K_{s1}+S_1}\right)\end{aligned} \tag{5.20}$$

To solve this, one must introduce the initial condition, using the relationship:

$$S_1(t) = S_1(0) - \frac{\int_0^t OUR_{ex}(\tau)d\tau}{1-Y_1} \tag{5.21}$$

yielding:

$$\begin{aligned}&\frac{\partial OUR_{ex}}{\partial S_1(0)} \\ &= \frac{(1-Y_1)\mu_{max1}X}{Y_1}\frac{\partial}{\partial S_1(0)}\left(\frac{(1-Y_1)S_1(0) - \int_0^t OUR_{ex}(\tau)d\tau}{(1-Y_1)\left(K_{s1}+S_1(0)\right) - \int_0^t OUR_{ex}(\tau)d\tau}\right)\end{aligned}$$

and the final equation:

$$\begin{aligned}&\frac{\partial OUR_{ex}}{\partial S_1(0)} \\ &= \frac{(1-Y_1)^2\mu_{max1}XK_{s1}}{Y_1}\left(\frac{(1-Y_1) - \int_0^t \frac{\partial OUR_{ex}(\tau)}{\partial S_1(0)}d\tau}{\left((1-Y_1)\left(K_{s1}+S_1(0)\right) - \int_0^t OUR_{ex}(\tau)d\tau\right)^2}\right)\end{aligned}$$

5.3 Optimal Experiment Design for Parameter Estimation (OED/PE)

We have already introduced the basic concepts of optimal experiment design (OED) in Chapter 1. Let us now concentrate on OED applied to parameter estimation. The quality of a set of parameter estimates can be assessed in different ways, e.g. in the way they allow a model to make good predictions of process behaviour. However, in most cases, one specifies parameter estimation quality by providing information on the parameter estimation errors (confidence intervals as given in (5.7)) or more generally, by providing the covariance matrix (5.5) altogether. Clearly the quality of parameter estimation is directly related to the practical identifiability of parameters.

If the objective of an experiment design exercise is to improve parameter estimation, it is evident that this covariance matrix (5.5) or elements thereof, or its inverse, the Fisher Information Matrix (5.4), are a central component.

Although we will extensively discuss the methods built around these matrices, other methods to deal with practical identifiability problems may be more appropriate. Indeed, sometimes the route of improving the experimental data is not followed to improve identifiability. For instance, it is sometimes possible to transform the model into an equivalent form that is numerically more tractable leading to more reliable estimation [208]. Alternatively, one may take a more drastic step and leave the initial model structure for a reduced order model that is less "data-hungry" and with improved practical identifiability [134]. It has also been proposed to use additional a priori information – such as a known maximum growth rate – to impose parameter bounds and in this way improve the identifiability of the other parameters [179].

One may also try to circumvent a practical identifiability problem by changing the goal of the modelling exercise. Indeed, sometimes it may suffice only to give a reasonable description ("curve fit") of the experimental data. Dedicated estimation algorithms, such as the set membership [143] or GLUE [28] methods have been developed that yield sets of parameters that allow description of process "behaviour". The goal of the modelling exercise is then no longer to find unique estimates but one is content with a set of good parameter values.

5.3.1 *Theoretical Background of OED/PE*

If one aims at designing experiments for optimal parameter estimation (OED/PE), it is illustrative to recall that the variable describing the reliability of a parameter estimate, the approximate standard error of the parameter, is given by:

$$\sigma(\theta_i) = s\sqrt{V_{ii}} \tag{5.22}$$

in case a single output variable is measured for parameter estimation purposes.

One observes two terms that can be manipulated to increase the parameter estimation accuracy. The first one, the residual mean square s^2 is readily calculated from the sum of squared errors between N model predictions and experimental data J_{opt}:

$$s^2 = \frac{J_{opt}(\theta)}{N - p} \tag{5.23}$$

To a certain extent this term can be decreased by increasing the number of experimental data N, e.g. by repeating the experiment. This is especially useful when p is not negligible compared to N. When N is already large, the increase of the denominator will be proportional to the increase of the objective functional J_{opt} as this is typically a sum of N squared errors.

Alternatively one may aim at reducing the parameter estimation error covariance matrix V. The methods to decrease V by optimal experiment design are the focus of attention in this section.

Different strategies have been developed to design experiments in such a way that the measurement data allow unique determination of the (combinations of)

parameters that were shown to be structurally identifiable, i.e. produce "informative" experiments. The Fisher Information Matrix F or, equivalently, the covariance matrix V are the cornerstones of the optimal experiment design procedures because these matrices summarise the information content of an experiment or the precision of the parameter estimates. Depending on the requirements imposed by the application different scalar measures of these matrices are optimised [180]:

$$A-optimal\ design\ criterion: \quad min[tr(F^{-1})] \tag{5.24}$$

$$Modified\ A-optimal\ design\ criterion: \quad max[tr(F)] \tag{5.25}$$

$$D-optimal\ design\ criterion: \quad max[det(F)] \tag{5.26}$$

$$E-optimal\ design\ criterion: \quad max[\lambda_{min}(F)] \tag{5.27}$$

$$Modified\ E-optimal\ design\ criterion: \quad min[\frac{\lambda_{max}(F)}{\lambda_{min}(F)}] \tag{5.28}$$

in which $\lambda_{min}(F)$ and $\lambda_{max}(F)$ are the smallest and largest eigenvalue of the Fisher Information Matrix.

The following interpretation can be given to these optimal experiment design criteria [180]. The A- and D-optimal designs minimise the arithmetic and geometric mean of the identification errors respectively, while the E-criterion based experimental designs aim at minimising the largest error. Because in these criteria a maximisation of eigenvalues of the Fisher Information Matrix is pursued, they guarantee the maximisation of the distance from the singular (non-informative) case. The modified E criterion should be interpreted in the frame of the objective functional shape. The ratio of the largest to the smallest eigenvalue is an indication of this shape. The objective is to have eigenvalues as close as possible to each other: the shape is then circular. When $\lambda_{min}(F)$ is zero, this ratio is infinite, i.e. an infinite number of parameter combinations can be used to describe the experimental data and, hence, the experiment is non-informative. The Fisher Matrix is then singular, and, hence, the D- and E-criteria are zero while the A-criterion cannot be determined since inversion of F is impossible. This case also points to problems that can be encountered with the modified A-criterion: even if a non-informative and unidentifiable experiment is conducted, the modified A-criterion may still be maximised because one of the other eigenvalues has become large [103].

Petersen [193] pointed to a property of the Fisher Information Matrix that is very relevant to experiment design. Inherently the elements of the Fisher Information Matrix are dependent on the unit of the parameters. For instance, the unit of the diagonal elements of the Fisher Information Matrix is the square of the unit of the parameter this matrix element corresponds to. Consequently, the eigenvalues of the Fisher Matrix are unit dependent and can therefore be manipulated by rescaling the units. Henceforth, an experiment that is optimal for one particular set of units, may not be optimal for a rescaled parameter estimation problem. This is true for all experimental design criteria, except for the D-criterion: although the

absolute value of this criterion is different for different parameter units, the optimal experiment remains the same. Hence, only for the D-criterion is the optimal experiment scale-invariant. To make her point particularly clear, Petersen [193] proved that the best attainable value of the Modified E criterion (Modified E=1) could be obtained simply by adequate rescaling of the parameters. This result has quite some implications for the experiment design methodology developed around such scale dependent criteria.

On the other hand, as mentioned before, the unit dependency of the Fisher Information Matrix can be used to the advantage of parameter estimation in case numerical problems occur with its inversion. Indeed, simple rescaling of the parameter units can change the condition number (which is equivalent to the Modified E criterion) and therefore the reliability with which inversion of the matrix can be done. This is particularly relevant for quite a number of numerical optimization algorithms used for parameter estimation (see Chapter 6).

Finally, it should be mentioned that other design criteria can be proposed, e.g. reducing the estimation error of a particular parameter can be obtained by designing experiments with this particular variance component as design criterion.

5.4 Examples of OED/PE

Below are a few studies which review the design of experiments allowed to collect more informative data. Vialas *et al*. [276] proposed to sample more frequently in defined periods of the experiment whereas Holmberg [126] showed that the practical identifiability of Monod parameters from batch experiments depends significantly on the initial substrate concentration. This author further stated that the optimal initial substrate concentration depends on the noise level and the sampling instants. It is also obvious from her results that the experiment design is dependent on the parameter values, which, in view of the changing nature of the process studied in the wastewater treatment case study, implies that the experiment design is time-varying. Munack [179] proposed different modifications to batch experiments and it was shown that important improvements in parameter confidences can be achieved by optimal experiment design techniques.

Vanrolleghem *et al*. [258] reported enhancements in estimation accuracy for the two parameters of a Monod type biodegradation model. By adding an addition pulse of substrate to a batch experiment the confidence interval of the parameters could be reduced by 25%. This variance reduction was well-balanced over both parameters μ_{max} and K_S which is a typical result of a D-criterion based OED. This example is dealt with in great detail in the subsequent section to illustrate the different aspects of optimal experiment design for parameter estimation.

Petersen [193] performed a similar study for a full-scale application in which a wastewater biodegradation model was to be identified. The model contained two submodels, one for nitrogen oxidation (nitrification) and one for carbon oxidation.

Here too an improvement in parameter estimation accuracy was obtained by complementing the wastewater sample with a designed amount of ammonia. For the carbon oxidation submodel parameters the confidence intervals were 20% smaller whereas they were 50% smaller for the nitrification kinetic parameters.

Baetens *et al*. [10] estimated six parameters in a biological phosphorus removal process. Optimal experiment designs were evaluated with a number of degrees of freedom. The D-criterion could be improved with a factor of at least 4, but this was completely attributed to a change in the reactor's acetate concentration, as no effect of changing the phosphate concentration could be observed in the system under study. This result corresponds with an average improvement of the parameter estimation confidence intervals with a factor 2. The evolution of the E-criterion with changing experimental conditions indicated that the longest axis of the confidence ellipsoid could be reduced with a factor 2 since the E-criterion could be increased with a factor 4 by increasing the dosage of acetate.

In relation to the degrees of freedom for experiment design, Petersen [193] used another approach. She evaluated the differences in parameter estimation accuracy when different sets of measured variables were used to identify a nitrification model. The following possibilities were evaluated:

- Single dissolved oxygen measurement S_O in an aerated batch reactor
- Two dissolved oxygen measurements S_O at in- and outlets of a closed respiration chamber
- Respiration rate OUR_{ex} calculated from two oxygen measurements
- Proton production rate H_p obtained from the pH controller
- Two dissolved oxygen measurements S_O and the proton production rate H_p.

Despite the fact that for the design options with oxygen concentration measurements the mass transfer parameters K_La and $S_{O,sat}$ need to be estimated in addition to the three nitrification parameters μ_{max}, K_{NH} and $S_{NH}(0)$, the (nitrification parameter) estimates one is interested in can be estimated much more accurately when dissolved oxygen measurements are used, i.e. the confidence regions are no less than 10 times smaller (or the variance is 100 times smaller). The larger noise on the respiration rate measurements is a partial explanation of this difference. This result points out that it is important to carefully reflect on the measured variable one is using for parameter estimation.

Munack [180] also evaluated the effect of different measurement set-ups that could lead to significantly different information contents of the data sets. He looked not only at the type of measurements, but also at the number of them and their location within an aerated column reactor.

Versyck *et al*. [273] reported on a quite remarkable result in terms of optimal experiment design. For the more accurate estimation of the parameters in a Haldane type microbial growth model, a Modified E criterion based design of the

substrate feed profile was conducted. Starting from a feed profile that was optimal in the sense of process performance, the authors were able to reach the truly optimal value of the Modified E criterion, namely the value is 1.

Versyck and Van Impe [274] also reported on the design of a temperature profile to identify a temperature dependency model of microbial growth kinetics. Again the unicity of the Modified E criterion could be reached. In the work conducted by Versyck and co-workers attention was drawn to the fact that more than one experiment design leads to this truly optimal value. It is therefore possible to also take into account additional criteria to select among the different possible designs, for instance practical feasibility or model validity. The latter aspect was also pointed out by Baltes *et al*. [12].

5.5 Application: Real-time OED/PE in a Respirometer

5.5.1 *Degrees of Freedom and Constraints for OED/PE*

Designing identification experiments requires several choices, e.g. what outputs should be measured at what time instants and at what frequency, and what inputs to manipulate and how. In the case study the output (OUR_{ex}) and sampling frequency (6 min^{-1}) are no longer available to the experimenter since they are fixed by the respirometer hardware used in the study. The only degree of freedom left is the design of the input. Optimal experiment design therefore reduces, in this case study, to find the input functions $u(t)$ that lead to the most informative experiments.

First, if one only considers batch experiments, the only possibility to change the information content of the experiment is the initial condition as imposed by the pulse of wastewater sample injected at the start of the experiment [126]. However, considering the assumption that biomass is constant in the course of the experiment and the on-line character of the sensor (the maximum experimentation time is 40 minutes), a constraint is placed on the maximum initial substrate concentrations.

As Munack [179] pointed out, fedbatch experiments are superior to batch experiments with respect to the practical identifiability of model parameters. In the respirometer under study, this degree of freedom is available as well since the wastewater pumps can be activated at any time, providing additional wastewater pulses to the bioreactor. A constraint is imposed, however, on the amount of sample injected per pulse. Real-time constraints must again be taken into account for the experiment design.

In the sequel, four examples of OED/PE will be developed theoretically:

1. Optimal initial substrate
2. Optimal additional pulse with fixed initial substrate
3. Optimal additional pulse and initial substrate
4. Optimal design with multiple additional pulses

In addition, the first and second design option will be illustrated with real-life data.

5.5.2 *OED/PE for the Single Monod Model*

Introduction. In Section 4.6, the structural identifiability of four kinetic models (Exponential, Single Monod, Double Monod and modified IWA ASM1) was studied, based on OUR_{ex} measurements. Here, we concentrate on the practical identifiability and the optimal experiment design for parameter estimation (OED/PE) of one of these models (the Single Monod model):

$$\frac{dS_1}{dt} = -\frac{\mu_{max1} X}{Y_1} \frac{S_1}{K_{s1} + S_1} \tag{5.29}$$

$$OUR_{ex} = -(1 - Y_1)\frac{dS_1}{dt} \tag{5.30}$$

The choice of the Monod model is, at least partially, motivated by its very large use in biotechnological applications. More specifically in the context of this study, this choice means that it is assumed that the experimental data are characterised by Single Monod kinetics, either because the real-life data are always characterised by this type of kinetics, or because a preliminary model structure characterisation has been performed, leading to the selection of Monod kinetics.

It was shown in Section 5.6 that three combinations ($\frac{\mu_{max1} X (1-Y_1)}{Y_1}$, $(1-Y_1)S_1(0)$, $(1 - Y_1)K_{s1}$) of the five original parameters (μ_{max1}, X, Y_1, $S_1(0)$, K_{s1}) are structurally identifiable. In order to have a presentation as pedagogical as possible (via e.g. the use of 3-D plots of the confidence regions), it is assumed here that the initial substrate concentration $S_1(0)$, the yield coefficient Y_1, and the biomass concentration X are known a priori (e.g. via some separate experiments). This leaves two parameters (μ_{max1}, K_{s1}) to be estimated.

The start-up of a batch experiment by pulse injection of wastewater is included in the model via the initial conditions $S_1(0)$ (which will be the degree of freedom in the optimal experiment design). An additional term in the mass balance is required to describe the fedbatch experiments that are also treated in this section. In order to prevent numerical problems, a pulse injection of wastewater in the course of an experiment is described by a Gauss-like function:

$$S_{puls} e^{-\frac{(t-t_{puls})^2}{\sigma}} \tag{5.31}$$

in which t_{puls} is the time instant at which the pulse is given, σ is the width of the pulse and $S_{puls}\sqrt{\pi\sigma}$ is the total amount of substrate injected.

Reference Data Set. As a reference data set for the theoretical examples of optimal experiment design, a single Monod model simulated OUR_{ex} profile was calculated with the following parameter values:

$$X(0) = 4000\ mg/l,\ S_1(0) = 23\ mg/l \tag{5.32}$$

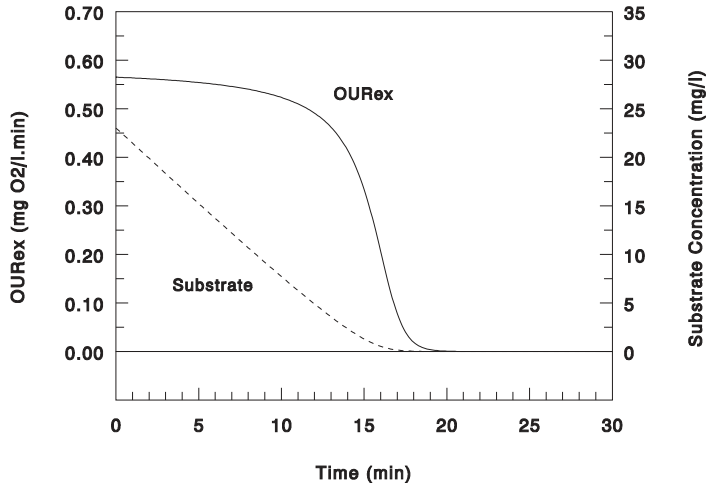

FIG. 5.3. Reference respirogram.

$$\mu_{max1} = 2.62\ 10^{-4}\ min^{-1},\ K_{s1} = 1\ mg/l,\ Y_1 = 0.64 \tag{5.33}$$

The resulting respirogram and corresponding substrate concentration trajectory are illustrated in Figure 5.3. The Fisher Information Matrix and the values of the different OED/PE criteria are equal to:

$$F = \begin{pmatrix} 3.456\ 10^{8} & -8182.2 \\ -8182.2 & 0.25702 \end{pmatrix},\ V = \begin{pmatrix} 1.175\ 10^{-8} & 3.742\ 10^{-4} \\ 3.742\ 10^{-4} & 15.802 \end{pmatrix} \tag{5.34}$$

$$tr(V) = 1.857\ 10^{-7},\ tr(F) = 8.882\ 10^{7},\ Det(F) = 2.186\ 10^{7}, \tag{5.35}$$

$$\lambda_{min} = 6.328\ 10^{-2},\ \frac{\lambda_{max}}{\lambda_{min}} = 5.46\ 10^{9} \tag{5.36}$$

Theoretical Example 1: Initial Substrate. We have looked for an optimal initial substrate concentration by using the different experiment design criteria introduced above. Figure 5.4 shows the different criterion values as a function of the initial substrate concentration.

For comparative purposes the optimal concentrations proposed by the other criteria are indicated in each graph. All criteria except for the modified E criterion tend to a batch experiment with almost 60 mg S_1/l as the initial concentration.

When considering the first four criteria, experiments are proposed with the highest possible information content with the aim of decreasing the variances of the estimates. This practically implies that the exogenous oxygen uptake rate is different from zero for the longest possible time. Hence, substrate is added initially in such an amount that it is not depleted until the allowed experimentation time (in this example 40 minutes). Confidences in the estimates of μ_{max1} and K_{s1} improve

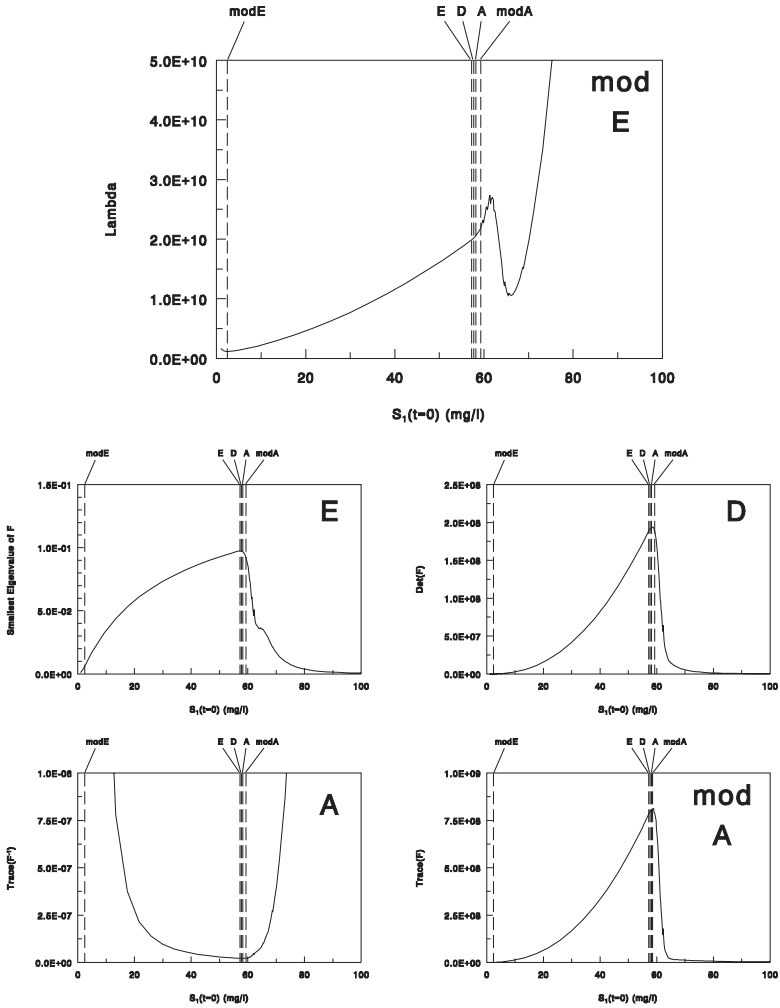

FIG. 5.4. Values of the OED/PE criteria with respect to the initial substrate concentration. Vertical lines indicate the optimal concentration for each criterion.

with a factor 2.4 and 1.25 respectively compared to the reference experiment. This indicates that experiment design with the initial substrate concentration as a degree of freedom is mostly beneficial to the estimation of the maximum growth rate.

The modified E criterion proposes an experiment with a very low substrate concentration of only 2.55 mg S_1/l. This can be interpreted as follows. In Figure 5.5 one can observe the flat valley for the parameter estimation problem of the reference OUR_{ex} profile. This flat valley is the main cause for the numerical

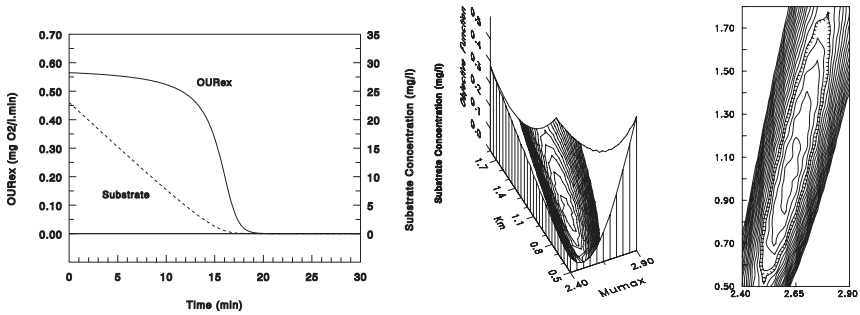

FIG. 5.5. 3D- (middle) and contour plot (right) of the objective function of the Monod model parameters for the reference respirogram (left).

problems related to the parameter estimation of Monod-type models. To improve the practical identifiability, the modified E criterion aims at OEDs where the objective functional's shape is as close as possible to a cone or funnel. The modified E based experiment that is obtained starts with a substrate concentration which is 10 times lower than the reference experiment. While the objective functional's shape has improved (the eigenvalues ratio has decreased by 3.3), the variances of the parameters indicate that this numerical advantage is at the expense of parameter estimation quality, i.e. the confidence regions have increased significantly, for μ_{max1} by one order of magnitude and for K_{s1} with a factor 3. This "improvement" (with respect to the modified E criterion) has been achieved by lowering the number of experimental data with a high sensitivity with respect to μ_{max1}.

Theoretical Example 2: One Additional Pulse. Let us now examine the effect of a fedbatch experiment on the practical parameter identifiability. If one considers that the pulse characteristics are fixed by the hardware used, i.e. pulse volume of the sample pump and mixing intensity in the reactor, the only degree of freedom to be evaluated here is the time of pulse addition, t_{puls}. In order to illustrate the increased flexibility more clearly, the initial substrate concentration is chosen identical to the reference case of the previous example.

Figure 5.6 illustrates the effect on the error functional's shape of an additional pulse of 8 mg/l given at the optimal time in a fedbatch experiment, according to the modified E criterion (t_{puls} = 18.2 min). The OUR_{ex} and substrate profiles of this experiment are given as well. One observes that, although still very 'valley-like', the properties of the error functional have been significantly improved (the eigenvalue ratio has decreased by a factor 3.5). A closer look at the covariance matrices V for the reference (no pulse) and optimal experiments for S_1(t = 0) = 23 mg/l:

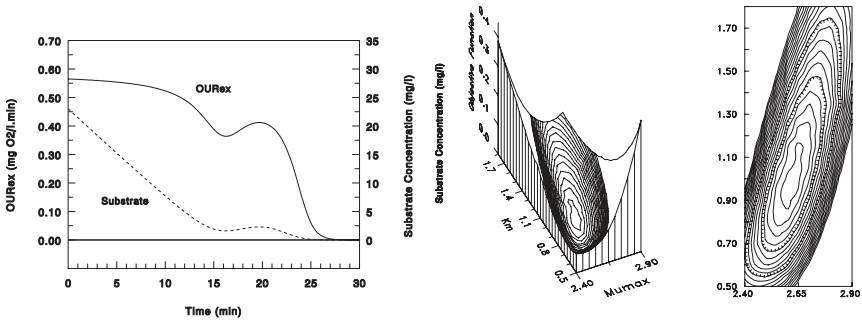

FIG. 5.6. 3D- (middle) and contour plot (right) of the objective function of the Monod model parameters for the respirogram with optimal substrate pulse (of 8 mg/l) at t = 18.2 min (left).

$$V_{Reference} = \begin{pmatrix} 1.175\ 10^{-8} & 3.742\ 10^{-4} \\ 3.742\ 10^{-4} & 15.802 \end{pmatrix} \tag{5.37}$$

$$V_{OED/PE} = \begin{pmatrix} 9.623\ 10^{-9} & 1.752\ 10^{-4} \\ 1.752\ 10^{-4} & 6.735 \end{pmatrix} \tag{5.38}$$

shows that all the variances and covariances have improved, but in contrast with the previous example, the OED with an additional pulse is especially attractive for a more accurate estimation of the affinity constant. Indeed, while the confidence interval for the μ_{max1} only decreased by 10%, the K_{s1} accuracy increased by more than 50%. In addition, the results show that the covariance between both biokinetic parameters is reduced almost to the same extent.

The study was also extended to evaluate the other OED/PE criteria. In this overall study, however, a pulse amount of 2 mg/l was taken. In Figure 5.7 the optimised OUR_{ex} and substrate profiles are summarised. As before the differences among the design criteria are considerable.

1. To optimise the A- and modified A criteria, the experimental conditions where maximal substrate degradation takes place are prolonged, a feature which was also noticed in the previous example. This can probably be explained by the fact that these criteria try to minimise the mean variance of the parameters. It may well be that this can be achieved by improving only one of the variances, and potentially one could have designs in which one variance improves to such an extent that the variance deterioration of another variance is compensated. With the sludge properties (5.32)(5.33), the most important improvement seems to be possible for the μ_{max1} parameter and, consequently, experimental conditions are proposed that take advantage of this.
2. For the D- and E-criteria, experiments are proposed in which a fresh amount of substrate is injected only after the exogenous respiration has dropped completely.

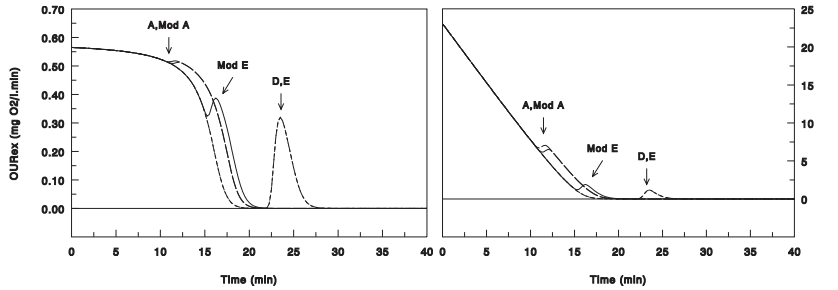

FIG. 5.7. OUR_{ex} (left) and substrate concentration (right) trajectories of fedbatch experiments with pulse additions (2 mg/l) at different injection times as proposed by different OED/PE criteria.

3. The modified E-criterion based OED results in a design which is in between both approaches.

With the D-, E- and modified E-criteria the substrate concentration is driven to remain for a longer period of time in the lower part of the Monod model. Consequently, additional information is obtained on the substrate range where the highest sensitivity with respect to the affinity constant is found.

From this observation it is clear why parameter accuracy has improved most for K_{s1} (see above). As a drawback to the D- and E-criteria it must be noted that the proposed experiments are significantly longer (approximately 30 %) than the other experiments, which should be considered in view of the real-time nature of the respirometer. Clearly, imposing a maximum experiment length will eliminate this problem but will result in suboptimal experiment designs that are a compromise between information content and experimentation time.

Theoretical Example 3: Additional Pulse + Initial Substrate. Let us now investigate whether the combination of the two degrees of freedom introduced above gives rise to an additional improvement in experimentation quality. This is clearly a two-dimensional optimisation problem: both the optimal initial substrate concentration and optimal time of pulse addition must be found within the time frame imposed by the real-time constraint.

To illustrate the results more clearly, the optimal $S_1(0)$ will first be sought for a pulse addition at 18.2 minutes, the optimal pulse addition time obtained for the case with $S_1(0)$ = 23 mg/l (see above). At the end of this section some comments will then be given on the global 2-dimensional optimisation result.

Figure 5.8 gives the evolution of the different OED/PE criteria as function of the initial substrate concentration. The covariance matrices corresponding with the different optimal designs are equal to (note the large differences in the covariance values!):

$$V_{reference} = \begin{pmatrix} 1.175\ 10^{-8} & 3.742\ 10^{-4} \\ 3.742\ 10^{-4} & 15.802 \end{pmatrix},\ V_{Mod-E} = \begin{pmatrix} 8.257\ 10^{-8} & 1.050\ 10^{-3} \\ 1.050\ 10^{-3} & 16.72 \end{pmatrix}$$

$$V_{A,D,Mod-A} = \begin{pmatrix} 2.402\ 10^{-9} & 1.434\ 10^{-4} \\ 1.434\ 10^{-4} & 18.10 \end{pmatrix},\ V_{E} = \begin{pmatrix} 9.650\ 10^{-9} & 1.761\ 10^{-4} \\ 1.761\ 10^{-4} & 6.731 \end{pmatrix}$$

Again, the optimal experiment designs are significantly different. Figure 5.8 exhibits local extrema corresponding to conditions that are optimal for other criteria, especially for the E-optimal experiment designs. On one hand, this looks rather reassuring: if the wrong criterion is chosen, still suboptimal experiments are performed with respect to the other criteria. On the other hand, this does not seem to hold for the D- and modified A criteria where the E-based design gives rise to a local minimum in information quality. The modified E criterion has a rather different behaviour compared to the others: low initial substrate amounts (7 mg/l) are proposed to optimise this criterion. This is similar to the behaviour observed to an even higher extent in the case where only the substrate concentration was available for design. This deviation from the other criteria is probably due to the different underlying objective, i.e. to improve the numerical properties of the error functional shape.

Another interesting result concerns the substrate concentration of 23 mg/l for which the additional pulse was optimised (see above). The E-criterion keeps this value as the optimal one. This initial substrate concentration corresponds to a secondary (local) minimum for both the modified E and the A-criterion. However, 23 mg/l is considered as a poor experiment design value for both other criteria.

The presence of local extrema in the criterion profiles illustrates the problems that may arise in looking for the global optimal experiment design. While it has not been documented for the designs in which even more degrees of freedom are available, it can be expected that attaining the globally optimal design may be difficult. The 2-dimensional design problem that is treated next may give a first indication of the expected problems.

In order to get insight in the dependency of the OED-criteria on the design variables, a grid was evaluated of substrate concentrations ranging between 1 and 40 mg/l and a pulse addition at times between 1 and 40 minutes after the start of the experiment. A total of 40×40 combinations were simulated. The results are summarised in the 3D-plots of Figure 5.9. The substrate and time for pulse addition that are optimal according to a criterion are marked on these figures. In Table 5.1, the improvement of the criterion values is compared to the values for the reference respirogram (with $S_1(0) = 23$ mg/l) and the values for the experiments in which the time of pulse addition was the only design variable. The gains in criterion values are important, but depend on the type of considered criterion.

A more detailed analysis indicates that the initial substrate concentration is maximised within the limit of 40 mg/l as imposed by the grid choice, except for the modified E criterion which proposes lower substrate concentrations as before,

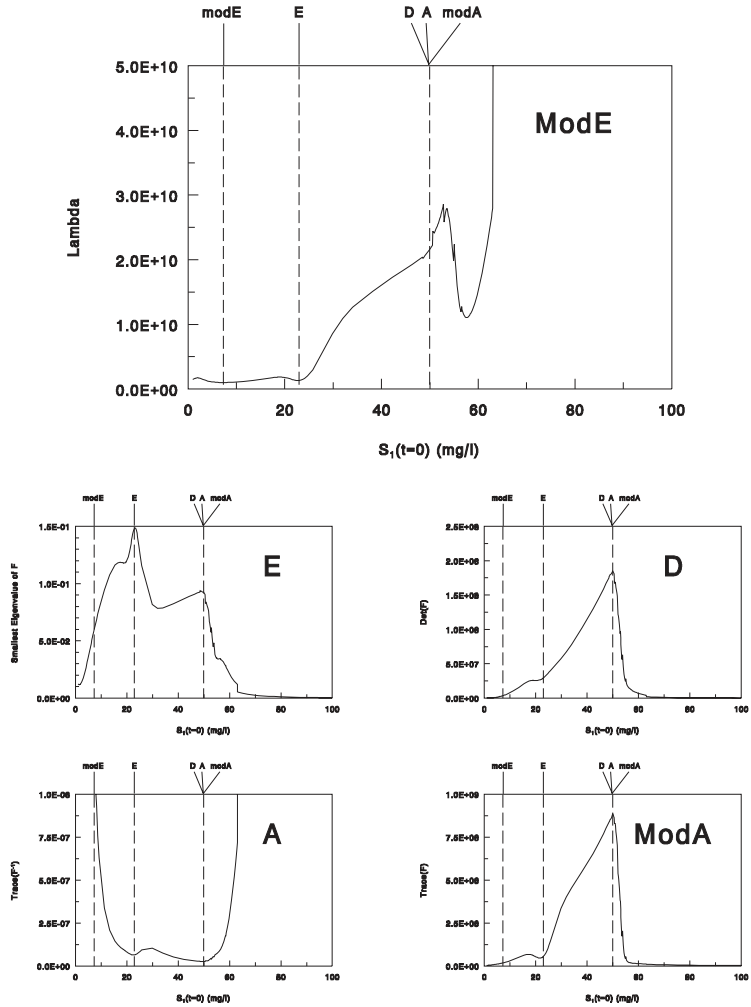

FIG. 5.8. Values of the OED/PE criteria with respect to the initial substrate concentration in a fedbatch experiment (t_{puls} = 18.2 min). Vertical lines indicate the optimal concentration for each criterion.

sacrificing μ_{max1} estimation accuracy to obtain a more cone-like error functional shape. All but the modified A criterion propose to inject the additional amount of substrate after 36 minutes. Clearly, this value is influenced by the 40 minute limit of the experiment, since to obtain the full information of the extra OUR_{ex} peak requires that the decreasing part of this peak finishes before data collection stops. This feature is visible in all 3D-plots where the criterion values decrease when t_{puls} exceeds 36 minutes.

Table 5.1 Optimal experiment design results with both the initial substrate concentration and time of pulse addition available for the design

	OED/PE		Gain in criterion values compared to	
Criterion	$S_1(0)$	t_{puls}	Reference	Optimised pulse
Modified E	4	36	7	2
E	40	36	2.05	1.65
D	40	36	5.8	1.65
A	40	36	7.75	2.2
Modified A	40	22	4.4	1.3

If one compares the 3D-plots (Figure 5.9) with the graphs in Figure 5.8 (that are in fact sections of the volume along the t_{puls} = 18.2 min line) the following observations can be made. A ripple on the surface (indicated with an arrow) can be found for all criteria. This corresponds to conditions in which the pulse addition is performed at the time the substrate initially present in the reactor is depleted. The experiments with "ripple conditions" result in OUR_{ex} profiles similar to the one presented in Figure 5.7, but with different lengths of the batch phase depending on the initial substrate concentration. For the modified E criterion surface, the valley is distinct but cannot be considered to be the minimum along any t_{puls} or $S_1(0)$ section. One can deduce it also from Figure 5.8: the minimum at 23 mg/l is only a secondary minimum. One finds for the ridge in the E criterion functional that the corresponding experiment designs are the optimum in the lower $S_1(0)$ range. At higher initial concentrations, however, the secondary (local) optimum becomes more pronounced and eventually takes over from the "ridge extremum".

Theoretical Example 4: Multiple Pulses. A next evident optimisation step is to consider experiment designs with multiple pulses of substrate addition. The obvious question is then whether the quality of the data is consistently improving and to what extent the marginal increase decreases.

In Figure 5.10 the evolution of the modified E-criterion as a function of increasing experimental freedom is depicted. One observes the decreasing effect of adding another degree of freedom to the experiment design.

A remarkable result of this case study is that the design can be performed sequentially: first, the optimum time for the first addition is determined; then, the next pulse time is optimised with this 1-pulse experiment. The simulation results indicate that the alternative optimisation of both pulses in one step gives only a minor improvement of 1.1 % in criterion value. The same conclusion was deduced when the design of a 3-pulse experiment was performed in a single or three optimisation stages. The sequential design has the important advantage that the computational burden is considerably lower since only one-dimensional optimisation problems must be solved.

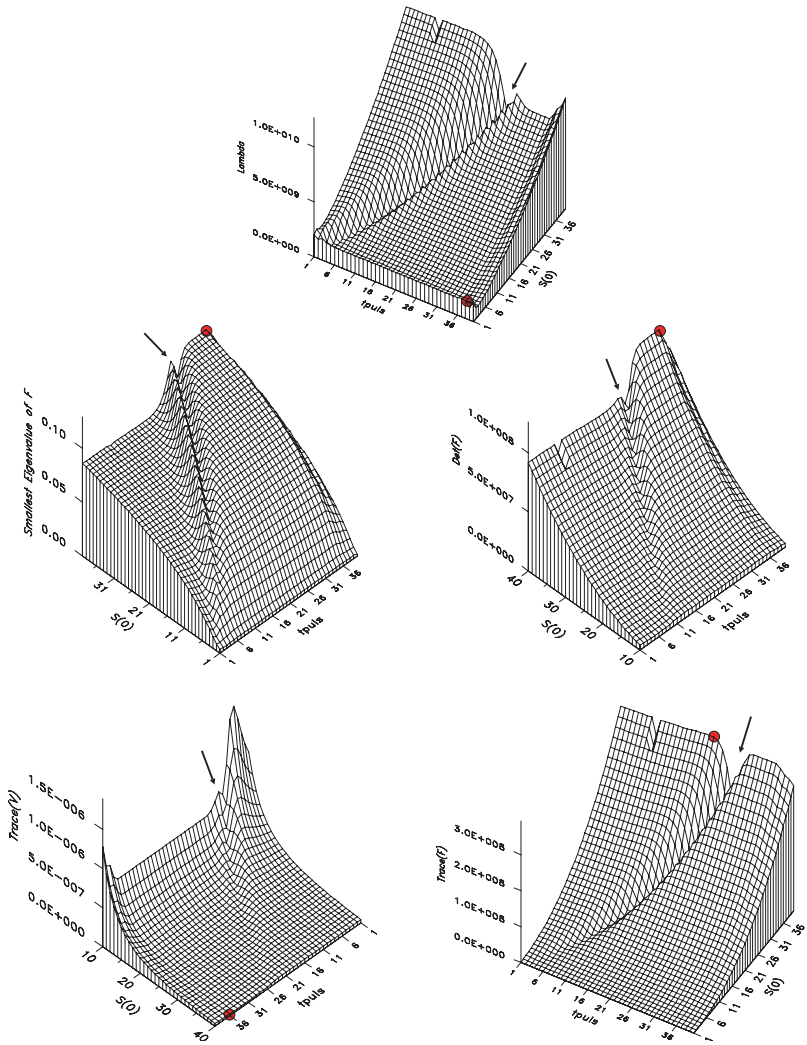

FIG. 5.9. Values of the OED/PE criteria with respect to the initial substrate concentration and time of pulse addition. Optimal experimental conditions are indicated with a circle, "ripple" conditions with an arrow.

This feature of the optimisation problem makes it even conceivable, to a certain extent, to adapt the experiments while they are still running, using the data obtained so far to decide about the quality of the experiment and to possibly add another pulse if necessary (see also [182]). Again one has to look for a compromise between accuracy and real-time constraint.

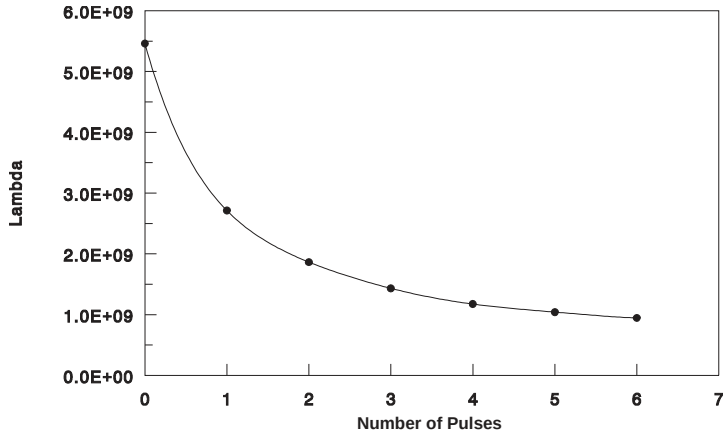

FIG. 5.10. Modified E criterion with respect to the increasing experimental flexibility.

A simulation of the optimal experiment obtained with six pulses is given in Figure 5.11. The numbers in the figure indicate the sequence in which the pulses are proposed by the OED method. One observes that the first two pulses are proposed to be injected during the decline phase of the OUR_{ex}. Adding two other degrees of freedom to the experiment design gives rise to pulses 3 and 4 that are initiated when the substrate is completely removed from the mixed liquor. If one allows two more pulses in the design (numbers 5 and 6), then these are scheduled such that the transients of pulse 3 and 4 are increased so as to enhance their information content.

Discussion of Theoretical Examples. The results presented above can be interpreted and summarised as follows:

- The information quality of the experiments is highly dependent on the design and major improvements (see Table 5.1) can be achieved by changing initial substrate concentrations and extending the experiments to fedbatch operation (with injection of additional substrate at an optimal time in the course of the experiments).
- It was observed that the different OED/PE criteria mentioned above yield different OEDs. The constraint imposed by the desired real-time operation of the respirometer is shown to be necessary since all but the modified E criterion would lead to prohibitively long experiments.
- More than one pulse addition further improves practical identifiability but the benefits become marginal as the experiment complexity increases. One or two additional pulses are apparently enough in terms of practical identifiability improvement.

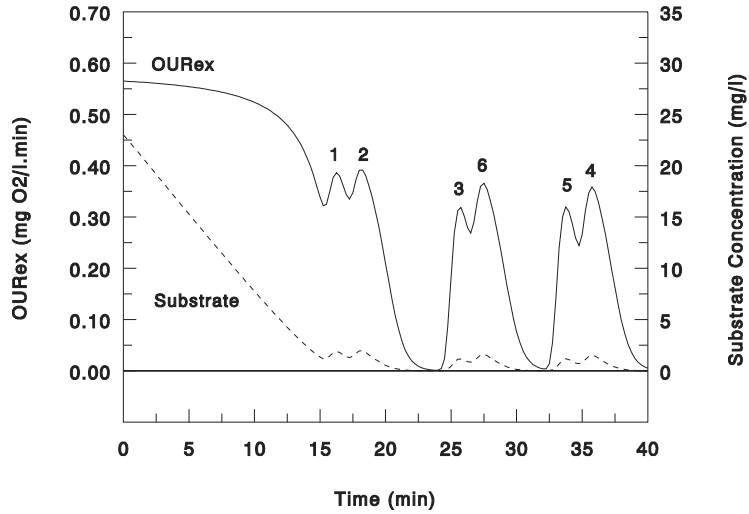

FIG. 5.11. OUR_{ex} and substrate concentration trajectories of fedbatch experiments with six pulse additions at injection times as proposed by the modified E criterion.

As a reasonable compromise of experimentation length and informative quality of the experimental data, it is proposed to perform respirometric experiments in which an additional pulse of substrate is injected at the time when the exogenous oxygen uptake rate is substantially decreasing, i.e. when the substrate has dropped to concentrations near to the affinity concentration. The amount of substrate at the beginning of the experiment is imposed by the allowable experimentation length.

5.5.3 *Experimental Validation of OED/PE for the Single Monod Model*

The above theoretical OED/PE results were checked in respirometric experiments performed with activated sludge that had slightly different properties than the sludge used in the theoretical OED/PE. First, a reference respirogram is commented and then the improvements obtained in parameter estimation accuracy are reported for improved initial substrate concentration and pulse additions.

Reference Experiment. The reference experimental OUR_{ex} profile consists of a batch experiment with an initial acetate concentration of 20 mg COD/l. Figure 5.12 presents the collected experimental data and the fit of the Single Monod model. The OED/PE study was mainly directed to the improvement of the numerical properties of the optimisation problem via experiment designs based on the modified-E criterion. Therefore, the objective functional's shape was calculated for a grid of parameter combinations μ_{max1}, K_{s1} in the neighbourhood of the optimum. It should be emphasised that the surface and corresponding contourplot depicted in Figure 5.12 is the result of systematic exploration of the error

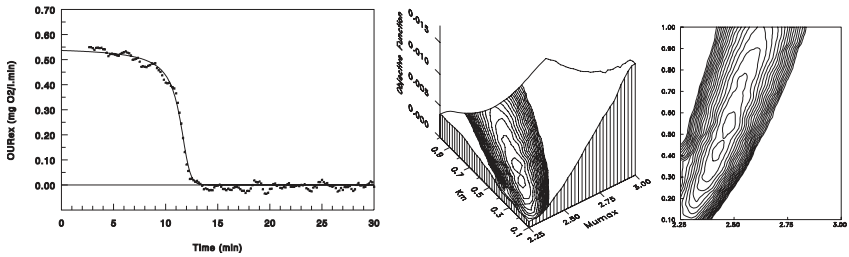

FIG. 5.12. 3D- (middle) and contour plot (right) of the objective function as a function of the Monod parameters for the validation reference respirogram (left).

functional in parameter space and is not a mere representation of the linearised objective functional around the optimum as it is often found in the literature ([161], see also Chapter 6).

This example of a flat valley in the parameter space may be the source of considerable problems to certain optimisation algorithms. The experience drawn from the cases studied so far tells that there exist adequate optimisation algorithms, such as the direction set method of Brent [42], which converge to the global minimum (μ_{max1} = 2.457 10^{-4} /min; K_{s1} = 0.456 mg COD/l). Still, the valley is undesirable and the aim of the study was to see whether the proposed OED/PE methods would result in improved properties.

The Fisher Information Matrix corresponding with this experiment and the deduced values of the different OED/PE criteria are summarised here below:

$$F = \begin{pmatrix} 3.475\ 10^{8} & -12250.1 \\ -12250.1 & 0.57715 \end{pmatrix},\ V = \begin{pmatrix} 1.148\ 10^{-8} & 2.443\ 10^{-4} \\ 2.443\ 10^{-4} & 6.926 \end{pmatrix} \quad (5.39)$$

$$tr(F) = 2.005\ 10^{8},\ det(F) = 5.03\ 10^{7},\ tr(V) = 7.95\ 10^{-8} \quad (5.40)$$

$$\lambda_{min}(F) = 0.145\ 10^{-2},\ \frac{\lambda_{max}}{\lambda_{min}}(F) = 2.3910^{9} \quad (5.41)$$

Experimental Validation of Example 1 – Initial Substrate. As a first validation test, the effect of a change in initial concentration on the error functional shape and the estimation accuracy is assessed. For this purpose a batch experiment was conducted with half the initial concentration of the reference experiment (see Figure 5.13). The modified E-criterion value calculated from the experimental results was 2.42 times lower than the reference value, i.e. lower substrate concentrations give rise to batch experiments in which the error functional is more cone-like. However, it was already pointed out that this numerical improvement is at the expense of estimation accuracy. Indeed, if the parameter variances are calculated, it is found that the variances increase, especially for the μ_{max1} parameter (increased with a factor 3.82) and to a lesser extent also for the affinity constant K_{s1} (a fac-

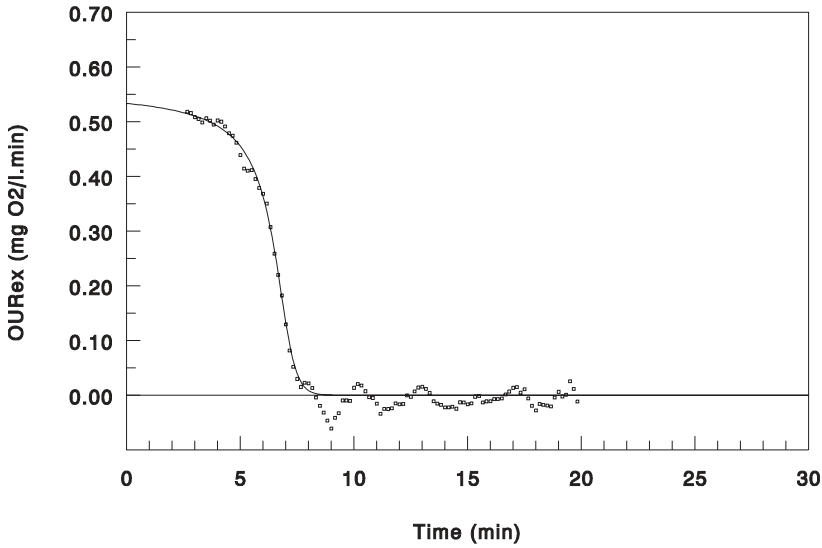

FIG. 5.13. Experimental respirogram in the initial substrate case.

tor 1.52). Let us note that OED based on the modified E criterion may sacrifice parameter estimation accuracy for improved numerical properties.

Experimental Validation of Example 2 – One Additional Pulse. The effect of an additional pulse of substrate was validated with three experiments in which the substrate concentration in the bioreactor was increased at some time instant with 2 mg COD/l. Different injection times t_{puls} were tested in order to illustrate the effect of an optimal t_{puls}.

Suppose first that the data of the reference example are available and that an additional experiment has to be designed with the possibility of adding one more substrate pulse. The calculations result in curves of criterion values versus injection time, summarised in Figure 5.14. These graphs show the differences in optimum injection time for the different design criteria. The A, D and E criteria lead to an optimal substrate pulse after 14.6 minutes (after complete degradation of the initially present substrate). The modified A criterion based experiment consists of a prolonged batch-phase. And the modified E criterion OED results in a respirogram in which the oxygen uptake re-accelerates just before complete disappearance of the initial amount of substrate.

Three experiments were performed with injection times of 13, 14.1 and 14.6 minutes respectively. The resulting OUR_{ex} profiles are given in Figures 5.15, 5.16 and 5.17.

A first important observation is that the model extension for fedbatch operation is capable of simulating the behaviour remarkably well. The pulse is described very

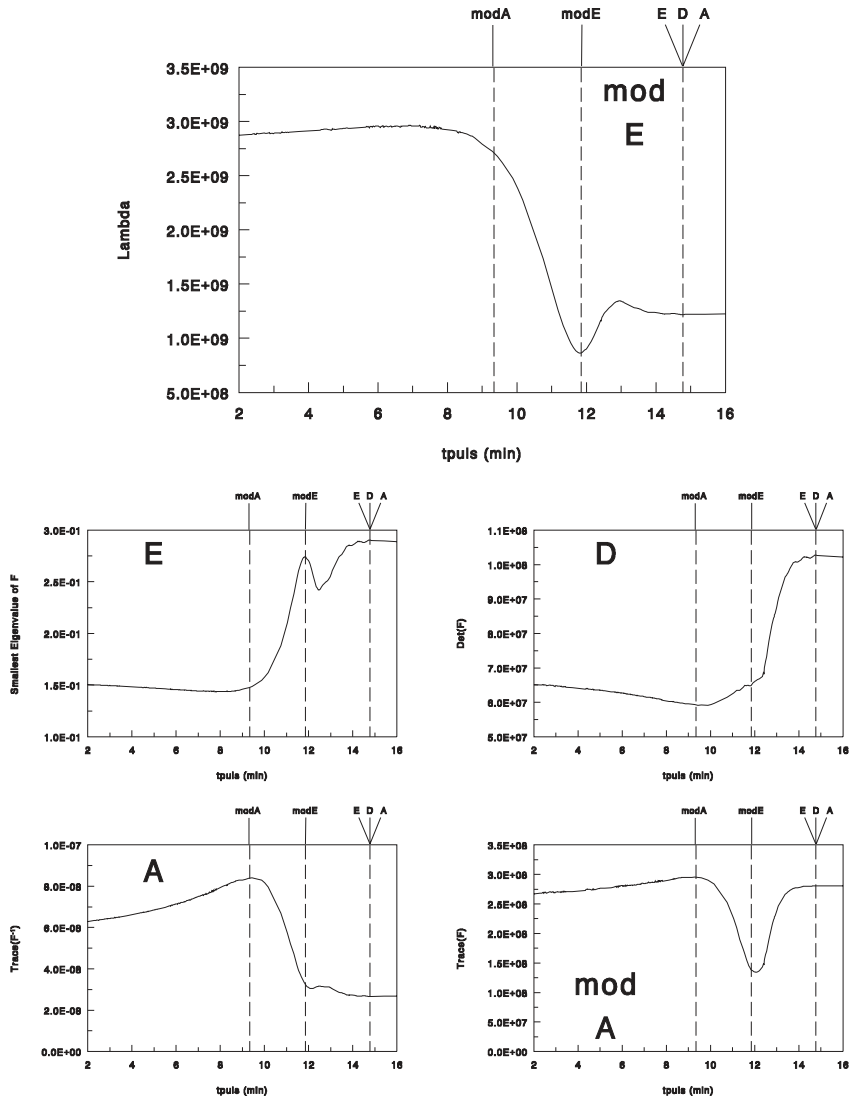

FIG. 5.14. Evolution of the different OED/PE criteria as a function of the pulse addition time (vertical lines = optimal time of addition for the different criteria).

well and microbial metabolism does not seem affected by the important transients imposed.

The following conclusions can be drawn when focusing on the effect of these fedbatch experiments on the error functional shape and parameter variances.

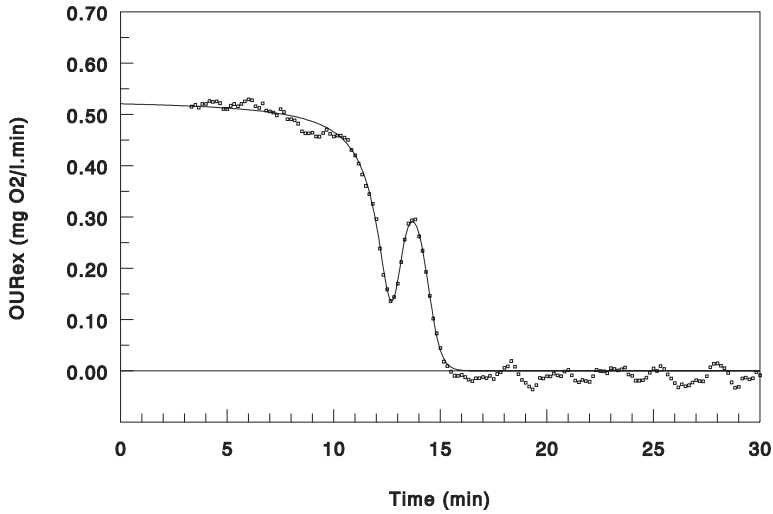

FIG. 5.15. Experimental respirogram obtained with a fedbatch experiment with additional pulse after 13 minutes.

Table 5.2 Dependence of the modified E criterion and of the parameter variances with respect to the pulse addition time

t_{puls}	Modified E	Var(μ_{max1})	Var(K_{s1})	Covariance
No pulse	1	1	1	1
13	0.676	0.4111	0.422	0.381
14.1	0.624	0.535	0.465	0.468
14.6	0.619	0.480	0.409	0.417

Although the expected values for the modified E criterion and the variances may change to a certain extent from the actually observed values due to changes in noise level, experimental error and biological changes, the trends set by the theoretical analysis are confirmed with these results. The predicted modified E criterion values for instance were approximately 20 % underestimated compared to the actual values. However, the data given in Table 5.2 clearly illustrate that a significant improvement in shape of the error functional is still obtained with fedbatch experiments. Moreover, as Figure 5.14 illustrates, the times of pulse addition that were evaluated were in the secondary minimum and more important effects could have been achieved if the substrate had been injected after 11.8 minutes.

A second conclusion concerns the variances. The experimental results confirm that significant improvements in parameter estimation accuracy can be obtained by this relatively small extension of the experiment. The variances have decreased with more than 50 % (Table 5.2). A similar effect on the parameter vari-

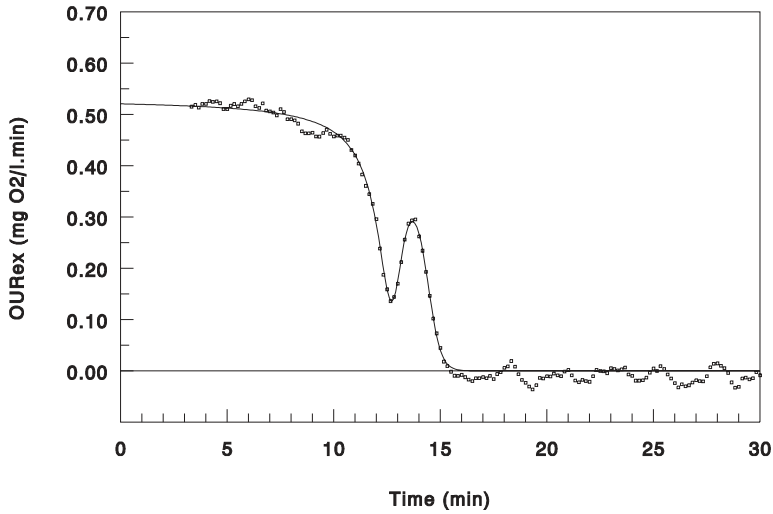

FIG. 5.16. Experimental respirogram obtained with a fedbatch experiment with additional pulse after 14.6 minutes.

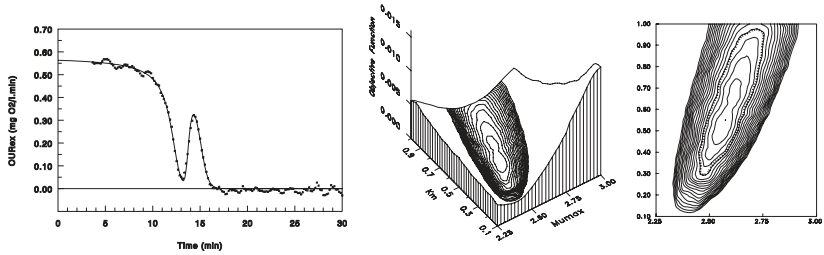

FIG. 5.17. 3D- (middle) and contour plot (right) of the objective function as a function of the Monod parameters for the validation respirogram with additional pulse after 14.1 minutes (left).

ance could also be obtained by repeating the experiment twice, recall (5.22)(5.23). However, this would double the experimentation time while the approach taken here increases the experiment duration by only 3 minutes, i.e. 10 % of normal operation.

5.6 Optimal Experiment Design for the Dual Problem of Structure Characterisation and Parameter Estimation

As it was presented so far, the goal of experiment design was geared to *either* parameter estimation *or* structure characterisation. Frequently, however, investigators

want to perform experiments that will shed light on both questions simultaneously.

The simplest approach, which is often intuitively taken, is to first apply the discrimination criterion to select the best model and then use an OED/PE procedure for design of experiments for reliable parameter estimation [123]. However, one can imagine that during the structure characterisation experiments important information is already collected on parameter values, and that, conversely, during the experiments for parameter estimation additional data are gathered on the most appropriate model structure. Most importantly, it is likely that the same model accuracy can be reached with fewer experiments if a joint design criterion were used.

The more integrated approach presented by Hill *et al*. [124] is based on a design criterion of the form

$$C = w_D D + w_E E \tag{5.42}$$

where D is a measure for the discriminative power of an experiment and E reflects the parameter estimation accuracy. Such a joint design criterion should emphasise discrimination among rival models when there is a substantial uncertainty as to which model is the best. It should also emphasise parameter estimation accuracy when one particular model of the model set is definitely superior over the others. Hence, the values of the relative weights w_D and w_E should gradually change as experimentation proceeds to reflect the relative emphasis that is given to each of the objectives. A sequential design procedure where an $(n+1)^{th}$ experiment is designed should therefore find the experimental conditions Ψ_{n+1} such that C is maximised. Hill *et al*. [124] have proposed a particular form for this joint criterion which is written as follows for the general case of m rival models:

$$C = w_D \frac{D}{D_{max}} + (1 - w_D) \sum_{j=1}^{m} \Pi_{j,n} \frac{E_j}{E_{j,max}} \tag{5.43}$$

where D_{max} and E_{max} are the maximum attainable values of D and E_j over the allowed Ψ-region. $\Pi_{j,n}$ is the probability of model j after n experiments, for which an iterative formula is given in [123]. The weighting w_D given to discrimination is suggested to be [124]:

$$w_D = \left(\frac{m}{m-1} \left(1 - \Pi_{max,n} \right) \right)^{\lambda} \tag{5.44}$$

in which $\Pi_{max,n}$ is the largest probability of the $\Pi_{j,n}$ after n experiments. The tuning parameter λ gives a degree of freedom to the experimenter on the rate at which interest shifts from OED/SC to OED/PE.

Very few results have been reported on the performance of such criteria. The example given by Hill *et al*. [124] indicates that no conflict arises as to the experimental conditions optimal for model selection or parameter estimation. In [123]

on the other hand it is mentioned that design conditions that are best suited for discrimination may not be optimal for accurate parameter estimation. Clearly, some more experience with such joint criterion is necessary.

5.7 Conclusions

In this chapter, we have added an essential brick in the model building exercise: the practical identifiability analysis, which is mainly concerned with the informative quality of the experimental data used for parameter estimation. More precisely, the practical identifiability is the capability to produce reliable parameter estimates with the available data. The natural complement of this concept is the generation of sufficiently informative data for parameter estimation, which has been called "optimal experiment design" for parameter estimation.

To improve the accuracy (combinations) of parameters that can be estimated (from a theoretical point of view at least), it was indicated that two possibilities exist. One is to repeat experiments, while the other consists of improving the quality of the experiment(s) performed. While the former is a straightforward approach, the latter was paid a lot of attention and it was illustrated using the case study developed throughout the text that important improvements in parameter estimation accuracy can be expected.

A similar optimal experiment design methodology was introduced in Chapter 3 for the goal of model structure discrimination. It was indicated that the main problem with the mentioned techniques concerned the lengthy computations necessary in case a posteriori structure characterisation methods are used for the model selection. With the case study, for which specific a priori SC methods had been developed, it was shown that optimal experiment design does not necessarily have to violate the time constraints.

In the last section of the present chapter attention was paid to the application of a joint experiment design criterion for model discrimination and parameter estimation. Because its use may substantially reduce the necessary experiments in case of an overall model selection exercise, this approach warrants more attention than given so far.

6

Estimation of Model Parameters

6.1 Introduction

So far we have spent our time and the pages of the book to build models (Chapter 2) and to give tools for analysing these models, either for structure characterisation (Chapter 3), for structural (Chapter 4) and for practical identifiability (Chapter 5). Now we finally arrive at the following practical questions:

1. We have built and/or selected a model and we have a complete set of appropriate data (preferably after optimal experiment design (see Chapter 5): how do we estimate the parameters of this model ?
2. We have a model with estimated parameters, and we intend to use it for following the time evolution of (some of) the state variables (typically, process component concentrations) and/or parameters[8] (e.g. maximum specific growth rates) from available on-line measurements (typically, liquid and gaseous flow rates and some component concentrations): Which tools are we going to use?

The first topic (en bloc parameter estimation (identification or calibration)) will be the object of the present chapter. Chapter 7 will be concerned with algorithms

[8]Note that a parameter was defined in Section 1.2.1 as a model constituent whose value is constant for a particular system under study. However, here we will also consider that such a system may be characterised by time-varying behaviour which results in "time-varying parameters". Strict on the definition of a parameter, it means that the model is rebuilt every time the parameter changes.

to estimate (track) state variables and parameters on-line as new data become available.
Parameter estimation is defined as

"the determination of the "optimum" values of the parameters that arise in a mathematical description with the aid of experimental data, assuming that the structure of the process model, in other words the relationships between the variables and the parameters, are explicitly known."

This definition should be read with some caution as some overlap between parameter estimation and structure characterisation may occur. Indeed, for some particular parameter values (typically for zero values) a "branch" of a model structure may be deleted. Therefore, following [240], it is assumed that all parameters have non-zero (or other particular) parameter values.

The chapter is organised as follows. We shall first give two simple examples to introduce the two types of estimations mentioned above. In Section 6.3 we discuss some preliminary steps to be taken in a parameter estimation problem, such as the parameters that can be considered for estimation, linearisation and reparametrisation of the model, how to obtain initial guesses for the parameters to be estimated and the way to deal with constraints on parameter values. Section 6.4 focuses on the characteristics of measurement errors as they will determine which method is to be used for parameter estimation. The next section deals with the different objective functions that should be used for different parameter estimation problems. In Section 6.6, some linear and nonlinear parameter estimation algorithms are introduced in a rather qualitative manner. It is important to realise that most of the algorithms presented are basically optimisation algorithms that can also be useful in other tasks than parameter estimation, e.g. optimisation of an experiment design. Finally, in Section 6.7 methods are given that allow the assessment of the quality of the parameter estimation procedure and, hence, the resulting identified model. It also adresses the question of the quality of the parameter estimates themselves and the generation of confidence information.

6.2 Introductory Examples

As we shall be looking at a number of aspects of parameter estimation, two simple examples are given to help

- to differentiate between the two major types of estimation, i.e. en bloc versus recursive estimation and
- to get a feeling for the problems nonlinearities in the model cause in parameter estimation.

6.2.1 *Example 1: Estimating the Mean of a Data Set*

Probably the best known estimator is the determination of the mean of a random variable on the basis of N samples. With this example, Spriet and Vansteenkiste

[240] illustrate three basic computation schemes for estimation problems, the en bloc, the recursive and the iterative method.

En bloc method. This method is applied when all the measurements have been completed at the time the computation is initiated. Straightforward calculations allow us to obtain the en bloc estimate:

$$\bar{y}_N = \frac{1}{N} \sum_{i=1}^{N} y_i \tag{6.1}$$

Recursive method. When one wants to be computationally efficient in case the current mean of a data set with increasing size is desired, the recursive approach is preferred. Indeed, this method allows the estimation of the mean of a growing data set with minor calculations each time a new data point is gathered. Computing recursively is basically characterised by two features:

1. The data set is expanding during computation;
2. Intermediate estimates of the quantity one desires are available and these values converge to the en bloc solution as the data set completes.

A recursive specification of the sample mean is the following:

$$\begin{aligned} \hat{a}_0 &= 0 \\ \hat{a}_k &= \hat{a}_{k-1} - \tfrac{1}{k} \left(\hat{a}_{k-1} - y_k \right) \end{aligned} \tag{6.2}$$

which is calculated for k increasing from 0 to N.

Note that the algorithm shows the major features of recursivity. First, during computation the amount of data used, increases:
for $k = 1$: data base $\{y_1\}$

$$\vdots$$

for $k = 2$: data base $\{y_1, y_2\}$
for $k = N$: data base $\{y_1, y_2, \ldots, y_N\}$

Second, a sequence of numbers is obtained:

$$\hat{a}_1, \hat{a}_2, \hat{a}_3, \ldots, \hat{a}_N$$

However, the second feature asks for a proof that $\hat{a}_N$ converges to $\bar{y}_N$. We do not do this formally, but show how the recursive estimate can be deduced from the en bloc calculation procedure:

Write the en bloc solution for $k - 1$ and k samples

$$\hat{a}_{k-1} = \frac{1}{k-1} \sum_{i=1}^{k-1} y_i \tag{6.3}$$

$$\hat{a}_k = \frac{1}{k}\sum_{i=1}^{k} y_i = \frac{1}{k}\left(\sum_{i=1}^{k-1} y_i + y_k\right) \tag{6.4}$$

and we readily obtain:

$$\hat{a}_k = \frac{k-1}{k}\hat{a}_{k-1} + \frac{1}{k}y_k = \hat{a}_{k-1} - \frac{1}{k}\left(\hat{a}_{k-1} - y_k\right) \tag{6.5}$$

The validity of the recursive procedure follows from induction. Although the en bloc (non-recursive and single iteration) method of estimating the mean is well-known, the recursive algorithm is relatively little known. And yet, the algorithm is significant in a number of ways: not only is it elegant and computationally attractive, it also exposes, in a most vivid manner, the physical nature of the estimate for increasing sample size, and so provides insight into a mechanism which is useful in many more general problems. Referring to the above equation, the previous estimate $\hat{a}_{k-1}$ is modified in proportion to the error between the observation of the random variable and the latest estimate of its mean value. Consequently, we will devote a complete chapter to this type of recursive algorithms, i.e. Chapter 7.

Iterative method. In the third computational technique, the whole data set is used sequentially to obtain a solution. Just as in the recursive method, a sequence of solutions is obtained that converges to the en bloc estimate:

$$\hat{a}_N^1, \hat{a}_N^2, \hat{a}_N^3, \ldots, \hat{a}_N^m$$

In contrast to recursion where the sequence is due to the increasing data set, the sequence created in the iterative method originates differently. In the iterative case, all data are available before the computation starts, but the en bloc method cannot be applied directly and it has to be approximated by successive calculations on the whole data set. Only the estimate is updated at each iterative step, not the data set.

It is clear that the iterative method is "overkill" for the estimation of the mean. However, to illustrate the point, let us assume that the algorithm which will compute the mean is very crude in carrying out divisions. To calculate (6.1), it will divide 1 by N approximately and then multiply $\sum_{i=1}^{N} y_i$. En bloc computation will yield an approximate value: $\hat{a}_N = \frac{\alpha}{N}\sum_{i=1}^{N} y_i$. Iterative processing can now be used to increase the precision of the sample mean estimate. Consider the following function:

$$f(x) = xN - \sum_{i=1}^{N} y_i \tag{6.6}$$

By definition, for $x = \bar{y}_N$, $f(\bar{y}_N) = 0$. An iterative scheme for finding a zero of a function is the Newton-Raphson procedure:

$$x_{k+1} = x_k - \beta\frac{f(x_k)}{f'(x_k)} \quad \text{with } 0 < \beta < 2 \tag{6.7}$$

For the problem at hand,

$$\begin{aligned} \hat{a}_N^1 &= \tfrac{\alpha}{N} \textstyle\sum_{i=1}^N y_i \\ \hat{a}_N^{k+1} &= \hat{a}_N^k - \tfrac{\alpha}{N} \left(\hat{a}_N^k \cdot N - \textstyle\sum_{i=1}^N y_i \right) \end{aligned} \tag{6.8}$$

where α/N represents the approximate division. The algorithm generates a sequence of approximations which converge to a precise value. Note that the complete data base is available and used all the time and that only the estimate is updated. For a proper use of iterative schemes a suitable stopping rule has to be provided, e.g.

$$\bar{y}_N = a_N^m \text{ for } m \text{ such that } \left| a_N^m - a_N^{m-1} \right| < \varepsilon \text{ or } m = m_{max} \tag{6.9}$$

For the purpose of parameter estimation, all the computational procedures presented in this section will be useful. The recursive methods will be dealt with in Chapter 7, whereas the two other methods are covered below.

Estimating the mean value: interpretations. Here above, only the computational aspects of estimating the mean have been dealt with, without specifying why equation (6.1) was proposed. The choice follows from classic estimation theory, which will not be discussed in detail here. However, the basic background is provided here.

First, we follow a statistical interpretation. If the random variable is Gaussian and if the samples are independent observations, then it can be proven that (6.1) is an optimal estimator in the sense that it has no bias and that its variance is smaller than for any other computational procedure. Under the conditions mentioned, equation (6.1) can be found as the maximum likelihood estimate. The recursive estimate (6.2) embodies a Bayesian point of view in the sense that with a priori knowledge of $\hat{a}_{k-1}$, a new sample y_k brings us to an a posteriori estimate $\hat{a}_k$. The concepts of maximum likelihood and Bayesian statistics will be shortly introduced in Section 6.4.

Second, we can look at the problem as a minimisation problem. When we define an objective function:

$$J(\bar{a}) = \sum_{i=1}^{N} (y_i - \bar{a})^2 \tag{6.10}$$

it is easily understood that minimisation of J corresponds to the search for a value that is in the centre of the samples. Note that no statistical assumptions are necessary here: all samples contribute to the objective function. The gradient per sample, also termed the instantaneous gradient, is $2(y_i - \bar{a})$. Equation (6.2), the recursive estimator, can thus be seen as a "gradient algorithm" in which the estimate $\hat{a}_{k-1}$ is

updated in a direction defined by the gradient of the instantaneous cost and with a magnitude of step size dictated by *1/k*, a weighting factor that is not constant but, in fact, is inversely proportional to the size of the data base at computation time. Thus, as the algorithm proceeds and confidence in the estimate increases, less and less notice is taken of the gradient measure, since it is more likely to arise from noise than from an error in the previous estimate of the mean value.

Third, reconsidering (6.10) we can obtain the en bloc solution of the mean by differentiating J with respect to $\bar{a}$, and setting it to zero:

$$\frac{\partial J(\bar{a})}{\partial \bar{a}} = 2\sum_{i=1}^{N}(y_i - \bar{a}) = 2\sum_{i=1}^{N} y_i - 2N\bar{a} = 0 \tag{6.11}$$

yielding the minimum of the objective function, i.e.:

$$\bar{a} = \bar{y}_N = argmin J(\bar{a}) = \frac{1}{N}\sum_{i=1}^{N} y_i \tag{6.12}$$

and, consequently, the en bloc solution (6.1).

6.2.2 *Example 2: A Simple Nonlinear Parameter Estimation Problem*

A nonlinear regression model is one in which the parameters appear nonlinearly, for example:

$$y = x^{\theta} \tag{6.13}$$

where θ is the parameter to be estimated. The fact that the model is nonlinear in θ can easily be checked by taking the partial derivative of the right hand side with respect to θ and conclude that the result is still a function of θ.

Let us now estimate the parameter θ using the same objective function as in example 1 above, i.e. the least squares objective function

$$J(\theta) = \sum_{i=1}^{N}(y_i - \hat{y}_i)^2 = \sum_{i=1}^{N}(y_i - x_i^{\theta})^2 \tag{6.14}$$

where $\hat{y}_i$ is the prediction of the model output at sampling time instant i. The minimum of this objective function can be obtained by differentiating (6.14) with respect to θ, setting the derivative to zero, as follows:

$$\frac{\partial J(\theta)}{\partial \theta} = -2\sum_{i=1}^{N}(y_i - x_i^{\theta}) ln(x_i) x_i^{\theta} = 0 \tag{6.15}$$

and attempting to solve for θ, the solution which is denoted $\hat{\theta}$. However, it appears impossible to obtain a nice explicit relationship as found in the en bloc method

for the estimator of the mean, equation (6.1). Instead, the resulting rearranged equation:

$$\sum_{i=1}^{N} y_i ln(x_i) x_i^{\hat{\theta}} = \sum_{i=1}^{N} ln(x_i) x_i^{2\hat{\theta}} \tag{6.16}$$

can yield the estimate $\hat{\theta}$ only by an iterative procedure starting for some assumed initial guess of $\hat{\theta}$. At this point, it is important to realise that most parameter estimation problems we will tackle in wastewater treatment models will be nonlinear in the parameters and will therefore depend largely on iterative solution methods that may be computationally demanding. Parameter estimation is indeed a time consuming task.

6.3 Preliminary Steps in Parameter Estimation

Before we introduce the actual estimation of parameters as a solution of a minimisation problem, some preliminary activities that can or must be conducted at the start of a parameter estimation exercise are reviewed.

As mentioned in the introduction of this book, the quality of a model is assessed by a validation step. For this validation (or better, corroboration) model predictions are often compared to a data set that is not being used for identification. Some criteria that are useful to divide an overall data set in an estimation and validation subset are mentioned. Next, we will discuss the selection of parameters that will be estimated, leaving the others fixed to certain (assumed) values. Different methods that support this selection are reviewed. Since linearity of parameters makes their estimation considerably easier, much work has been devoted at finding linear parameters and trying to estimate them separately. Moreover, models have been rewritten (transformed, reparameterised) in such a way that originally nonlinear parameters become linear in the new model formulation. Some examples of this are given as well and commented upon. In most algorithms for parameter estimation initial guesses of the parameter values must be given to start the iterative search procedure. The proper choice of these initial guesses is dealt with as well because they may determine whether the parameter estimation is successful. On some parameters physical or user-defined (inequality) constraints apply. Although constrained optimisation algorithms are able to deal with such problems, they are not covered in this chapter. Rather, alternative ways to include constraints in parameter estimation are introduced.

6.3.1 *Selecting Data Subsets for Estimation and Validation*

One of the most important preparatory steps in a parameter estimation exercise is to split the available data in two subsets:

1. A first set of data for estimation of the parameters;
2. The remaining data for validation of the model.

The first set of data will be used to calculate the estimates of the parameters using an appropriate estimation algorithm. The second data set will be used to verify that the model with these parameters is able to describe or predict the dynamics of the process (other aims of the model may yield different definitions of the objectives of the validation step). The independence of these two data subsets is fundamental for reliable validation. We may indeed expect that a model identified on the basis of a particular data set is able to reproduce these data well, but it is much more important to know whether this model is able to reproduce quite different data that have not been used for calibration of the parameters.

The separation of the data must be done in such a way that the first data set (for calibration) is sufficiently informative and covers a sufficiently large spectrum of experimental conditions (using for instance optimal experiment design methodology). The remaining data, on the other hand, must still contain sufficient data to allow for a validation that is as credible as possible. Hence, one will prevent to split the data in subsets that are unbalanced in terms of number of data, although typically the first (calibration) data set is larger than the second (validation) data set.

6.3.2 *Selection of Parameters to be Estimated*

We shortly refer to the important distinctions that have to be made here between constants, parameters and variables (see Section 1.2.1). In this chapter we will only deal with parameter estimation, as variables will be calculated by the model or given as time series whereas constants are assumed to be given from prior knowledge as they apply, by definition, to any situation that can be modelled. Henceforth, only parameters must be estimated for the particular application at hand.

In the context of parameter estimation, it is important to realise that initial and boundary conditions of state variables and some inputs can also be formulated with the aid of parameters. Consequently, the set of parameters to be considered in the parameter estimation problem contains all of these and can be estimated simultaneously.

Selection of the parameters to be estimated is an important starting activity in a parameter estimation exercise. Below we introduce a number of criteria to select a certain subset of model parameters: structural and practical identifiability analysis, sensitivity analysis and numerical properties of the estimation algorithms.

Structural identifiability analysis. Structural identifiability analysis allows you to find out the possibly identifiable parameters or combinations thereof (provided the data are sufficiently rich in information, see Chapters 4 and 5). Hence, only the structurally identifiable subset will be contained in the parameter estimation problem. Note that for any identifiable parameter combination, e.g. in Section 4.6.2, $(1-Y)*\mu*X/Y$, the following is allowed: Choose one of the three parameters to estimate (e.g. μ) and set values for the other two. When the parameter μ is now es-

timated, it means that its value found is conditioned by the choice of the values for the other two. Still, in case other values of the non-estimated parameters would be adopted, one does not need to repeat the estimation (of μ in the example) because one can always directly recalculate the corresponding value of the parameter that was estimated (in the example it was μ) by ensuring that the value of the parameter combination is maintained, i.e.:

$$(1-\bar{Y})\frac{\bar{\mu}\bar{X}}{\bar{Y}} = (1-\tilde{Y})\frac{\tilde{\mu}\tilde{X}}{\tilde{Y}} \tag{6.17}$$

Note that the above is something different than a model reparameterisation (see below) where the combination above would get a new "name" and associated meaning. This, for instance, has been done in [44] when the maximum growth rate parameter combination of the ASM1 model $\mu_A X_{BA}/Y_A$ was reparameterised into the maximum nitrification rate $r_{NH,max}$.

Practical identifiability analysis. When practical identifiability problems are encountered, for instance, because the data are insufficiently informative to reliably estimate all parameters, a subselection of this parameter set can be made after an analysis of the parameter estimation error covariance matrix (or its inverse, the Fisher Information Matrix), see Chapter 5. Indeed, by eliminating the parameter that is causing the identifiability problem from the parameter set and giving it an assumed value, the estimation of the other parameters may be highly facilitated. Note that the estimates of these parameters will then be conditional on the assumed value of the non-identifiable parameters. Beck [23] proposes to adopt this scheme for any parameter estimation problem, i.e. first the estimation is performed for all parameters and an analysis is made of the parameter covariance matrix. Those parameters with the lowest confidence are then eliminated and a new parameter estimation exercise is launched. Despite its elegance, he also points to the contradiction of fixing the most uncertain parameters, since fixing its value gives the impression of having perfect knowledge on the parameter value. Detailed studies on the effect of this type of parameter space delimitation have been conducted in [268].

Sensitivity analysis. Weijers and Vanrolleghem [282] and Reichert and Vanrolleghem [215] developed methods based on sensitivity analysis to preselect parameter subsets that ensure reliable estimation. However, these methods are computationally demanding due to the need to calculate the sensitivity functions (no attempt was made to do this analytically as in Chapter 5 in view of the complexity of the models to which the methods were applied). The applications made so far indicate that the two methods are leading to quite similar subsets of identifiable parameters. Furthermore, the fear existed that the identifiable parameter subsets would be highly dependent on the parameter values themselves (due to the nonlinear nature of the models this could be possible). If true this would mean that the

selection of identifiable subsets should be done for each application of a model for which the parameter values were different. Luckily, for the cases studied by the authors the subsets of identifiable parameters turned out to be hardly dependent on the parameter values considered. Still, one should remain cautious in this matter.

Numerical properties of the estimation problem. For numerical reasons it can be useful to restructure the overall parameter estimation problem in a stepwise procedure since a series of smaller parameter estimation problems are typically easier to solve than a single large one. For instance, one may first estimate the stoichiometric parameters before the kinetic parameter estimation is initiated. Another example may be that one first estimates the parameters related to the slow processes (decay, hydrolysis, nitrifier growth) and then the ones related to the fast processes (heterotrophic growth, aeration, ...). This type of stepwise parameter estimation typically induces a structuring of the data too. For instance, in case of the stoichiometric/kinetic parameter estimation split-up, typically long term averages of the data are taken for estimation of the stoichiometric parameters, whereas the more dynamic time series is used for estimation of the kinetic parameters.

In this stepwise approach it is important to realise the following. When the overall estimation of p parameters is started, we look for the best parameters in a p-dimensional parameter space. When we split the estimation problem into two sequential estimation problems, we will first estimate p_1 parameters and, given these p_1 parameter estimates, we will try to estimate the $p - p_1$ remaining parameters. However, it means that these $p - p_1$ other parameter estimates are conditional on the p_1 first estimates, possibly leading to biased estimation of the parameters. Combining the advantages of step-wise estimation with unbiased parameter estimation therefore leads to the following approach: first, the step-wise procedure is performed, but the estimates obtained are subsequently used to initiate an overall parameter estimation exercise where the search for the truly optimal estimates is done without any reduction in search space. Because the initial parameter estimates obtained from the stepwise procedure can be expected to be located relatively close to the overall best estimates, numerical problems are less likely to occur.

6.3.3 *Differentiating Linear and Nonlinear Parameters*

An important aspect of the models we try to identify is that many parameters appear in them in a nonlinear way. In Sections 1.2.2 and 2.6 we have introduced the definition and features of (non)linearity. Let us just recall that one of the easiest techniques to evaluate whether a function is nonlinear in a parameter is to take the partial derivative of the function with respect to that parameter and check whether the result is still a function of that parameter [218]. If the answer is affirmative the model is nonlinear in that parameter.

Below we will see that nonlinearity complicates the estimation problem considerably. It can therefore be advantageous to separate the linear from the nonlinear

parameters and create subsets of these two types of parameters. Indeed, methods have been developed that can split the estimation problem in two, i.e. a sequence of a less complicated nonlinear and a fast/simple linear estimation procedure, rather than a single, more complicated, nonlinear parameter estimation. A nice example of this parameter set partitioning is the RAWN method for efficient neural network training [246].

In Section 2.6 another important aspect of nonlinear parameters was drawn to attention. We shortly repeat the example here. Consider the simple model (2.211-2.212):

$$\frac{dS}{dt} = DS_{in} - DS - \frac{1}{Y}\mu_{max}X \tag{6.18}$$

$$\frac{dX}{dt} = -DX + \mu_{max}X \tag{6.19}$$

This model is nonlinear in the parameters Y and μ_{max} (division by Y and "multiplication" μ_{max}/Y). However, it was shown that by transforming the parameters with

$$\theta_1 = \frac{1}{Y}\mu_{max},\ \theta_2 = \mu_{max} \tag{6.20}$$

the model can be rewritten in a form linear in the parameters θ_1 and θ_2. Such transformation is also termed reparameterisation [208], [209] and is a powerful method to reduce (or completely eliminate, as shown above) the estimation complications due to parameter nonlinearity. Its main drawback is that the physical meaning of parameters may be lost. Note, however, that in the example above θ_1 still has a well-known meaning in wastewater treatment modelling: it is the maximum specific substrate uptake rate.

Nonlinearity is not only a yes/no property of a model. Different levels of nonlinearity can be discerned and methods have been developed to determine the level of nonlinearity of, for instance, a parameter estimation problem. Pioneering work was done by Beale [18] and Bates and Watts [17] and a good introduction of nonlinearity assessment is given by Ratkowsky [208]. From a parameter estimation point of view it is important to minimise the level of nonlinearity so as to maximise the quality of a parameter estimation result. Below we will introduce several parameter estimation complications (bias on the parameter estimates and incorrect prediction of parameter confidence information) that are due to nonlinearity but which have not too large effects on the estimation results as long as the level of nonlinearity is small.

6.3.4 *Use and Misuse of Linearised Forms of Nonlinear Models*

It appears to be common practice to transform a model that is nonlinear in the parameters into a model with linear parameters. It is important to note that the "linearisation" discussed here is not based on a Taylor series approximation, but is

really a transformation with no loss of accuracy. There are many models, though, that cannot be algebraically converted into linearised forms.

The best known example of such linearisation is probably the Lineweaver-Burk expression that transforms the Monod-type kinetic equation into a linear form:

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \cdot \frac{1}{S} + \frac{1}{\mu_{max}} \tag{6.21}$$

The rationale for pursuing this type of transformation is that many people involved in dynamic modelling still lack good education of nonlinear parameter estimation but do have good notion of linear regression. This allows them to efficiently obtain estimates of the parameters (in fact, estimates of the parameters are calculated from new parameters appearing in the linearised version of the originally nonlinear model).

This ease of analysis is, unfortunately, accompanied by fundamental drawbacks. When data are transformed (e.g. in the Lineweaver-Burk expression we transform S into a new variable 1/S and μ into $1/\mu$), the measurement errors are transformed too. More particularly, although the actually measured variables may be characterised as iidN (*independent and identically distributed normally*), the transformed variables will typically not be. Moreover, error-free independent variables may no longer occur in the transformed equation, leading to an errors-in-variable problem (see the example below on the Eadie-Hofstee transformation). Tseng and Hsu [251] call this the destruction of the error structure.

As mentioned in the forthcoming sections on error characteristics and parameter estimation objectives, the type of errors determines which objective function should be applied. Consequently, if the wrong assumption is made on the error characteristics, biased parameter estimates can be expected. It was indeed found by many authors that different linearised forms of the same nonlinear model yield different estimates of the same parameters [218], [190].

Problems in addition to biased estimates are the difficulties one encounters when trying to get hold of the precision of the estimated parameters. Indeed, the parameters that are actually estimated are not the parameters one wants to estimate, but typically combinations thereof. For instance, in the Lineweaver-Burk approximation, the parameters that are estimated (and for which confidence intervals would become available, albeit probably wrong since the error structure is not as it should be for linear regression) are $\theta_1 = 1/\mu_{max}$ and $\theta_2 = K_S/\mu_{max}$. It is not trivial to calculate the confidence information on μ_{max} and K_S given confidence information on θ_1 and θ_2.

A final drawback of using linearised forms is that they typically require more data points to achieve the same accuracy of the parameter estimates. Also, the spacing of the data becomes important. For instance, when applying the Lineweaver-Burk approximation, many data points are located at low values of the variable

(1/*S*) and only few are found at large values of (1/*S*), leading to a very high sensitivity of the parameters (especially the slope of the linear regression) to the latter values.

Related to the above example, we want to point out that transformation into a linear form can allow proper, unbiased estimation of the parameters using linear regression when the proper weighting is given to the different data points. For instance, Cornish-Bowden [62] reminds us of the original approach of Lineweaver and Burk in 1934 where the data of μ were weighted with μ^4 in the weighted regression performed. It appears that this aspect is not passed on when the Lineweaver-Burk method is taught.

In some cases a transformation into a linear form may happen to transform the error structure into one that is closer to the so desired iidN that would allow OLS estimation. This will be a lucky coincidence though. However, it must be stressed that it may be wrong to generalise that transformation into a linear form is helpful or harmful. It may produce either effect. Which one is applicable depends on the error structure of the data and not on the model.

A final appropriate and even recommended use of the transformation into a linearised form is the use of it as an easy means of obtaining the initial guesses for parameters. These guesses are needed to start a nonlinear parameter estimation algorithm (see below).

6.3.5 *Reparameterisation of Models with Nonlinear Parameters*

Using a multitude of examples Ratkowsky [208] has pointed out to what extent the nonlinearity of parameters in a model can cause significant errors in the estimates, even if the measurement error structure is considered adequately and the proper objective function is selected (see above). These errors are caused by the fact that the estimators used in nonlinear regression can be badly biased, non-normally distributed and have variances greatly in excess of the minimum possible variance. However, as the number of data increases toward infinity, the bias diminishes, the distribution of the estimator becomes more normal and the excess variance decreases, thereby approaching more and more closely the condition for a linear model. Some nonlinear regression models approach the large-sample behaviour even in small samples and Ratkowsky [208] termed such models "close-to-linear" and advocated searching for such models for practical use. This search for new models can be done using what is called reparameterisation, i.e. the analytical reformulation of a model to obtain certain properties of the model.

The level of nonlinearity of a model[9], expressed for instance by the curvature measures of Bates and Watts [17], is one of the properties one wants to control.

[9]Note that the level of nonlinearity will also be a measure of the difference to expected between confidence regions of parameters calculated using a linear approximation versus the regions obtained with the method of Beale [18], see the section Evaluation of Parameter Estimation Quality.

The level of nonlinearity is composed of two terms, the intrinsic and the parameter-effects nonlinearity. It is the latter component one can influence by reparameterisation.

An example may illustrate the basic idea. Consider again the Monod kinetic model and its reparameterised version:

$$\mu = \frac{\mu_{max} S}{K_S + S} \quad \mu = \frac{S}{\theta_1 S + \theta_2} \tag{6.22}$$

It is noteworthy that the two parameters θ_1 and θ_2 are exactly the parameters that are estimated through linear regression in the Lineweaver-Burk linearisation method. It was pointed out by Ratkwosky (1986) that putting the parameters of this model in the denominator was the way to obtain a close-to-linear model. Note the difference between this approach and the Lineweaver-Burk method. Here the parameters are obtained using nonlinear parameter estimation methods, but with less nonlinearity induced bias, whereas in the Lineweaver-Burk method linear regression is used, but with quite major drawbacks in terms of bias and error properties. Ratkoswky (1986) also provides the ways in which the confidence information on the original parameters μ_{max} and K_S can be calculated given the standard errors on θ_1 and θ_2. Furthermore, he extends the approach of parameters-in-denominator reparameterisations for a class of kinetic expressions and also discusses the necessary weighting functions needed to deal with measurement errors that are proportional to the magnitude of the variable.

In general, no set of simple rules is available to find the proper reparameterisation of a highly nonlinear model. However, from histograms of the estimation results for each parameter separately, hints can be found on the proper reparameterisation. For instance, a histogram with a long right-hand tail (characteristic of a log-normal distribution) suggests replacement of the parameter in the model by the exponential of the parameter.

In summary, reparameterisation is useful to get closer to a linear estimation problem which has an undeniable beneficial effect on the statistical properties of the estimation problem but it is accompanied with a loss in "meaningfulness" of the new parameters. However, in this respect Ratkowsky [208] rightfully states that pH is a reparameterisation of the proton concentration that we got really used to.

6.3.6 *Initial Estimates of the Parameters*

Except for the (lucky) situation in which parameters can be readily calculated analytically from the objective function (e.g. in linear parameter estimation problems, see below), the minimisation algorithms used need initial guesses for the parameter values. The choice of a good initial guess can spell the difference between success and failure in locating the minimum or between rapid and slow convergence towards it [13]. Unfortunately, while we can prescribe algorithms for proceeding

from the initial estimates, we must rely heavily on intuition and prior knowledge in selecting the initial guess.

Let us first of all warn that we should not exaggerate the importance of this initial guess either. It may be quite useful to just start with the first value that comes to mind and see where the algorithms bring us. A second and most obvious option is the use of prior knowledge, i.e. previous work done by yourself or others.

When a number of parameters are linear in the model, it is not necessary to select initial guesses for them too, since their optimal initial values can be readily calculated given the initial guesses of the nonlinear parameters [13]. For instance, suppose the nonlinear model to fit to a data set is $y(t) = \theta_1 exp(-\theta_2 t)$. If we have the initial guess $\theta_2 = 6$, the initial guess of θ_1 can readily be found by solving the linear ordinary least squares problem of minimising $J = \Sigma[y_i - \theta_1 exp(-6t_i)]^2$. This can be done in one calculation step (see below).

However, the most fruitful means of obtaining initial guesses is to substitute the original estimation problem by a simpler one. The solution to this simpler problem can then serve as good initial guesses for the original one. For instance, Ratkowsky [208] devotes a complete chapter of his book on nonlinear regression modelling to specify initial guess calculations for nine nonlinear models. Clearly the current chapter is not the place to introduce such dedicated methods, but should shed some light on some general principles for obtaining initial guesses. Below we introduce some approaches that have been applied successfully [13].

Reparameterisation in a linear form. In this approach we try by means of a transformation of the variables, to come to a model formulation that is linear in the parameters (examples of this were given above). Although we know that the parameter estimation problem is no longer properly defined due to the loss of the error structure (see above), the parameter estimates obtained by, for instance, multiple linear regression (that does not need initial guesses), can be considered to be good initial guesses for the original parameter estimation problem.

Multistage estimation. This type of estimation splits the set of parameters in groups of auxiliary parameters that are estimated in sequence on the basis of different subsets of data. The original parameters are then calculated by combining the separately estimated auxiliary parameters and their values are used as initial guesses for the original parameter estimation problem. Let us illustrate this approach with a simple example in which we try to determine the parameters describing the temperature dependency of a conversion process rate:

$$r = kSe^{-\frac{E}{T}} \tag{6.23}$$

where r is the rate, T is the temperature, S the substrate concentration, and k and E are the kinetic parameters to be estimated. Suppose we have measurements r_i

for q different temperatures T_i. We can now use the data taken at T_i to estimate an auxiliary parameter K_i that combines the parameters k and E:

$$r_i = K_i S \qquad (i = 1, 2, \cdots q) \tag{6.24}$$

This estimation is, again, a simple linear regression problem. The estimated K_I can then be used (as "data") to estimate k and E in the linearised model (with its known statistical limitations)

$$log(K_i) = log(k) - E T_i \qquad (i = 1, 2, \cdots q) \tag{6.25}$$

The values obtained for k and E can then be used as initial guesses for the original estimation problem.

Model simplification. In many instances it is possible to see the model under study as constructed from several submodels in which various subprocesses are considered. For instance, we may regard the Activated Sludge Model No. 1 as composed of different (interacting) submodels such as decay processes, nitrification, hydrolysis. We can try to approach the final model through a sequence of simpler ones, in which various effects are neglected and the corresponding parameters suppressed. After parameter estimation with the simpler model, these are used as initial guesses for a next parameter estimation of a more complex model. Basically this is the approach used in many activated sludge modelling protocols where, for instance, first the decay, hydrolysis and nitrification parameters are estimated before the overall model calibration is tackled with the parameter estimates found in these separate parameter estimations as initial guesses [193] [195].

6.3.7 *Inequality Constraints on the Parameters*

In many instances we know from prior knowledge that parameters are bound within a certain interval. For instance, by definition, all Monod half-saturation constants are non-negative, $K_S \geq 0$. Setting inequality constraints allows the domain of parameter values within which the estimate is to be found to be limited. The presence of inequality constraints often exerts a beneficial influence on the convergence of a minimisation algorithm since the algorithms are no longer "lost" in irrelevant regions of parameter space. It could therefore be argued [13] that imposing generous, though not unreasonable, bounds should be recommended in all parameter estimation problems.

The minimisation algorithms we will be discussing in the next section are the more traditional unconstrained minimisation algorithms. However, they are quite powerful and we would like to use them to solve constrained problems too. To that end we will have to modify our definition of the minimisation problem. Alternatively, we may turn to constrained optimisation methods, but we will not introduce these here as it would lead us too far (for the interested reader, reference is made to, for instance, [87].

Basically two approaches have been used to modify the minimisation problem. In the first one the objective function is extended by a term that penalises leaving the feasible parameter domain. In the other approach the interval of allowed parameters is projected onto the complete real axis via an appropriate transformation, ensuring that unconstrained minimisation in this new parameter domain guarantees constraining the original parameters.

Penalty functions. In this approach we modify the objective function in such a way that it remains almost unchanged in the interior of the feasible domain, but increases drastically when one of the parameters approaches the constraints. The approach of penalty functions is simple: for each inequality constraint $g_j(\theta) \geq 0$ we just add a term to the objective function to be minimised:

$$J_{constrained}(\theta) = J_{unconstrained}(\theta) + \sum_j J_{penalty,j}(\theta) \tag{6.26}$$

This penalty function is typically nearly zero when the constraint function g is strongly positive, but increases sharply as the constraint function approaches zero from above. A typical penalty function proposed by Carroll [48] is:

$$J_{penalty,j}(\theta) = \frac{\alpha_j}{g_j(\theta)} \tag{6.27}$$

where α_j is a small positive constant ensuring $J_{penalty,j}$ to be small compared to $J_{unconstrained}$ when in the feasible domain. To find the parameter estimates that minimise the original objective function $J_{unconstrained}$ and at the same time fulfill the constraints, an iteration over the value of α_j is necessary. Each time a minimum is found in $J_{constraint}$ with a certain α_j the estimates are used as initial guesses for a minimisation with an objective function with decreased value of α_j. This iteration is continued until the parameter estimates no longer changes significantly upon reduction of α_j.

The most pronounced penalty function is also called "barrier" function. It boils down to setting $J_{penalty,j} = \infty$ (or, practically, a very large number) whenever a parameter gets a value outside the feasible domain. The problem with this "barrier" approach is that a search algorithm that gets into the unfeasible domain is unable to find a direction back into the feasible domain as no gradient down can be found. To overcome this problem, it is suggested to linearly increase the "high cost function value" in function of the distance that a constrained parameter drifts away outside its boundaries. In other words, the high cost function plateau is replaced by a high cost function steep hill with a positive slope. The latter method was implemented by Van Vooren [269] and was found to perform without any problems.

The penalty function is easy to program and has been found to work well when the solution is known to be in the interior of the feasible domain. However, when the solution is likely to be on the boundary, other methods should be used (see for instance [13] or [87]).

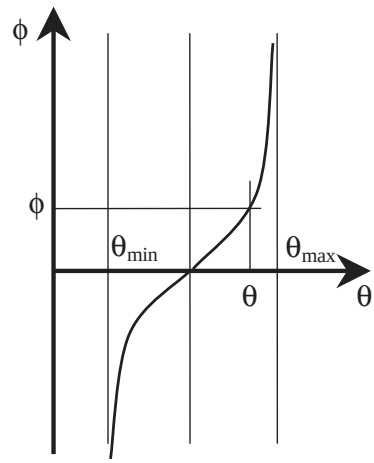

FIG. 6.1. Scaled tan-function used to transform the bounded interval $[\theta_{min}, \theta_{max}]$ of the parameter θ into the whole real axis for the parameter ϕ.

Transformation of parameters. A constrained minimisation problem can be transformed into an equivalent unconstrained one by transforming the parameters bounded in a feasible domain to the real axis by transforming the parameters. For instance, to minimise $J(\theta)$ with θ to be positive is equivalent to minimising $J(\phi^2)$ with ϕ free to assume any value. Bard [13] also mentions the transformation of a minimisation problem where we wish $\beta + \theta \geq \alpha$ into a minimisation of $J([\alpha + \beta]/2 + [(\alpha - \beta)/2]sin[\phi])$ where ϕ can again assume any value.

Another example of a useful parameter transformation is the mapping by a scaled *tan* function (corrected from [213]): The parameter θ within the interval between θ_{min} and θ_{max} is mapped to the whole real axis by the transformation (Figure 6.1):

$$\phi = tan\left(\frac{\pi}{2}\frac{2\theta - \theta_{max} - \theta_{min}}{\theta_{max} - \theta_{min}}\right) \tag{6.28}$$

The minimisation with the unconstrained algorithm can now be performed in the coordinates ϕ and the solution in the original coordinates is obtained by the inverse transformation:

$$\theta = \frac{1}{2}(\theta_{max} + \theta_{min}) + (\theta_{max} + \theta_{min})\frac{arctan(\phi)}{\pi} \tag{6.29}$$

which maps the real axis to the interval between θ_{min} and θ_{max}.

Schuetze [226] complained about this approach by stating that the minimisation algorithms he used were apparently not able to find a direction of improvement when searching close to the boundaries.

6.4 Error Characteristics

Although parameter estimation appears to be rather straightforward at first sight, i.e. we want to obtain values of the parameters that give the "best" fit to a given set of data, the definition of what is best requires considerable attention. To define "best" it is essential to characterise the errors one is confronted with during parameter estimation, i.e. measurement errors and model fitting residuals (the deviations between model predictions and data). After characterisation of these errors, a decision can be made on the objective function to be used in the parameter estimation exercise (Section 6.5) and only then the steps can be undertaken to prepare all inputs for the parameter estimation itself (Section 6.6).

6.4.1 *Measurement Errors and Residuals*

Assuming the selected model is a perfect representation of the system under study, the residuals between the model predictions $\hat{y}$ and the experimental data y are only due to measurement error. This error ε can be made explicitly visible in the standard model representation given in Section 1.2.1 (equations 1.7-8) as:

$$\begin{aligned} \frac{dx}{dt} &= f(x,t,u,\theta), \qquad\qquad x(t=0) = x_0 \\ y &= h(x,t,u,\theta) + \varepsilon(t) \end{aligned} \tag{6.30}$$

Hence, in parameter estimation we aim to find the parameters θ in such a way that the predicted residuals $\hat{\varepsilon} = y - \hat{y}$ possess properties that are similar to the properties one may expect of plain measurement errors, characterised e.g. via repeat measurements.

It is worth noting that this objective can typically only be reached when the model is adequate since otherwise model error is confounded with measurement error. Consequently, we have seen the development of model selection and validation criteria that focus on a thorough analysis of the residuals (see Chapter 3).

What are now typical properties of measurement errors? In many cases the errors are assumed to be "iidN", independent and identically distributed normally. In other words the errors are assumed to be random variables that are normally distributed with zero mean and constant variance (homoscedasticity) equal to one.

An example of a homoscedastic residual sequence is given in Figure 6.2(left). The distribution of the data in this illustration is not taken to be normal, but uniform. In the same figure on the right side a series of heteroscedastic (relative) errors is shown, i.e. the variance of these particular data increases along the X-axis.

6.4.2 *Autocorrelated Residuals*

Another important characteristic of errors concerns their serial dependency or autocorrelation. Figure 6.2 shows two residual series that are serially independent. Figure 6.3 on the other hand shows an experimental residuals series where autocorrelation is clearly apparent. The autocorrelation with time lag τ quantifies the dependency of a variable at any time t_k and the variable at time t_k-τ:

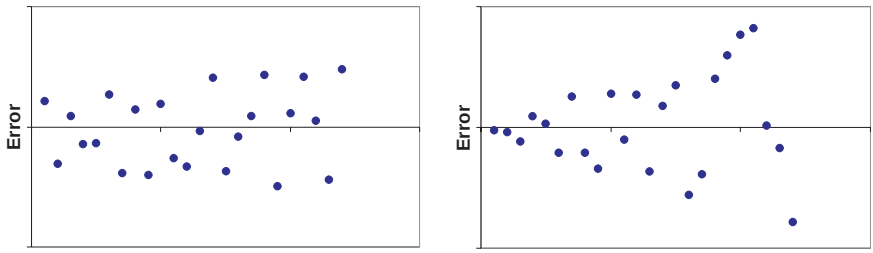

FIG. 6.2. Series of uniform homoscedastic (left) and heteroscedastic (right) errors.

$$r_\varepsilon(\tau) = \frac{1}{r_\varepsilon(0)} \sum_{k=\tau}^{N-\tau} \varepsilon(t_k - \tau) \cdot \varepsilon(t_k)$$

where $r_\varepsilon(0) = \sum_{k=\tau}^{N-\tau} \varepsilon^2(t_k)$

In the lower part of Figure 6.3 the autocorrelation for this data series is indeed found significant for 4 of the 20 first time lags (not considering time lag 0 because this always gives autocorrelation =1).

In Chapter 3 autocorrelation based tests for model selection were given. They allow to evaluate whether the residual sequence has the properties one may expect if the model is adequate, e.g. they should be independent if the measurement errors are. Another method introduced there concerned the runs test that also evaluates the dependency of residuals.

In this framework, an important feature to consider is that the N residuals obtained after fitting a model are in principle never uncorrelated (independent) because there are only N-p degrees of freedom left among them after estimation of p parameters [208]. Nevertheless, this violation of the assumptions is not really important and is therefore hardly ever focused upon.

In case autocorrelation is really significant, an approach that allows to still apply standard parameter estimation approaches is to correct for the correlated residual errors by including and identifying an autocorrelation model as part of the overall model [160]. Note that this approach complicates the modelling tasks because an autocorrelation model must be selected and its parameters estimated as well.

$$\begin{aligned} y(t_k) &= h(x(t_k), t, \theta, u(t_k)) + z(t_k) \\ z(t_k) &= \varepsilon(t_k) + \Phi(\varepsilon(t_{k-1})) \end{aligned} \tag{6.31}$$

Another approach consists in eliminating correlation from the data set by subsampling, i.e. by dropping data points from the raw data set. Evidently, information is lost in this way, but the principal estimation inaccuracies associated with correlated errors are eliminated. As an example, the autocorrelated data series given in Figure 6.3 are subsampled (retaining 1 in 6 values) and yield the non-correlated series of Figure 6.4

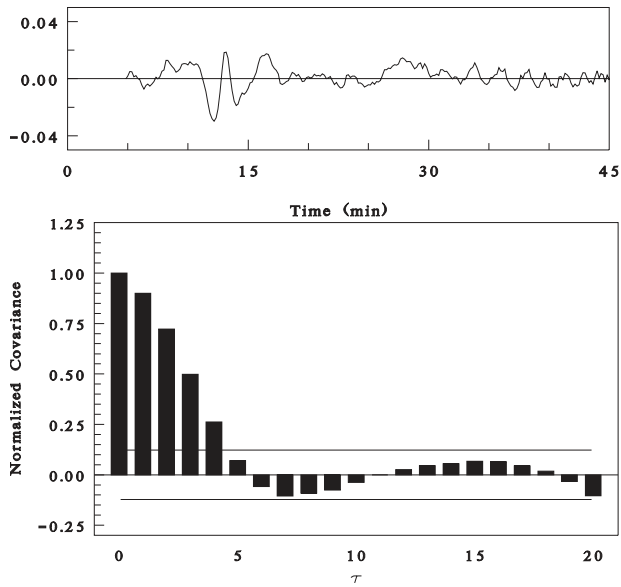

FIG. 6.3. Autocorrelated residual sequence (top) and autocorrelation for lags 0 to 20.

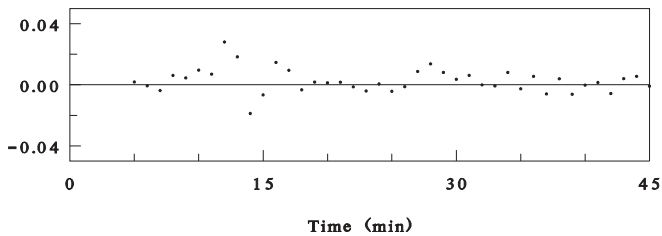

FIG. 6.4. Subsampled non-correlated data series of Figure 6.3.

6.4.3 *Estimation of the Measurement Error Covariance Matrix*

As will be more clear from the developments below, the determination of the measurement error covariance V is an important activity prior to many parameter estimation exercises. In the following example it will be described how the measurement error covariance matrix is estimated for a two variable data set collected in a combined respirometric-titrimetric set-up [193] [195]. The data series under study containing r_O (respiration rates) and *Hp* (protons produced) data are given in Figure 6.5.

Note that for neither of the two data series, repeat measurements are available for assessment of the measurement errors. Hence, another approach is required.

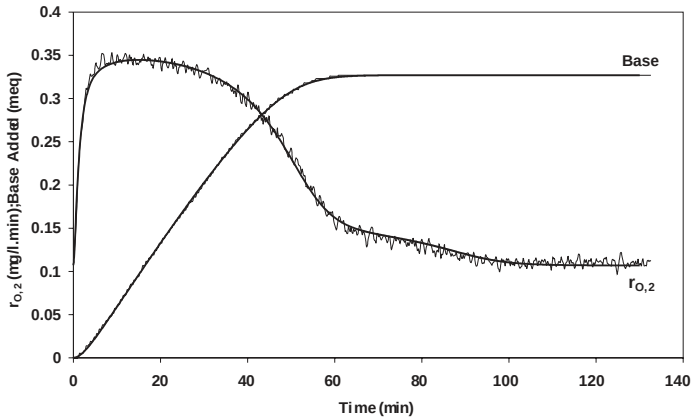

FIG. 6.5. Combined respirometric/titrimetric experimental data set [193] [195].

It is based on the selection of a period in which it can be assumed that the error remaining after fitting a (simple) model is pure measurement error and not containing modelling error.

For r_O data the measurement variance σ_O^2 is estimated based on a data series obtained during endogenous respiration (typically before or after the substrate addition). In the example (Figure 6.5) the measurement errors were estimated from t = 100-120 min where the model $r_O = r_{O,end} = constant$ was fitted. This selected data series is blown up in Figure 6.6 together with the average value and the residuals ($\varepsilon(t) = r_{O,end} - r_O(t)$). $\hat{\sigma}_O^2$ is estimated via equation (6.32) where N is the number of considered measurements and p is the number of adjusted parameters (here just 1).

$$\hat{\sigma}^2 = \frac{\sum_{i=1}^{N} \varepsilon_i^2}{N - p} \tag{6.32}$$

For the Hp data, however, an unrealistically optimistic picture would be obtained when the measurement variance were estimated at the point where substrate degradation is terminated. Indeed, the Hp profile in the example is a completely horizontal line with nearly no error due to the way the sensor operates [98]. As a consequence the σ_H^2 is estimated based on the data series from t = 15-35 minutes where, this time, the slope is assumed to be constant. Thus the data is not compared to an average value but to a model of the simple form $Hp = a*t+b$. The data series, model and residuals are illustrated in Figure 6.7. σ_H^2 is also estimated via equation (6.32), only now is p=2 (note that whether p is 1 or 2 does not really matter in these cases since $N >> p$).

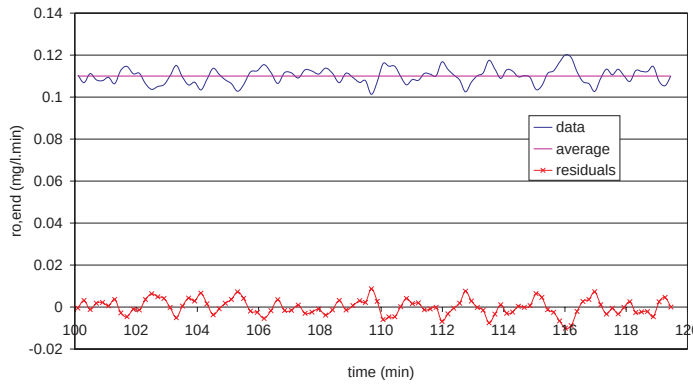

FIG. 6.6. Estimation of measurement variance on r_O data [193] [195].

To calculate the covariance between Hp and r_O data, σ^2_{OH}, the following calculation is made:

$$\hat{\sigma}^2_{OH} = \frac{\sum_{i=1}^{N} \varepsilon_i^{Hp} \varepsilon_i^{r_O}}{N - p} \tag{6.33}$$

The covariance among the residuals was estimated to be:

$$V = \begin{bmatrix} \sigma^2_O & \sigma^2_{OH} \\ \sigma^2_{OH} & \sigma^2_H \end{bmatrix} = \begin{bmatrix} 1.385 \cdot 10^{-5} & 2.541 \cdot 10^{-7} \\ 2.541 \cdot 10^{-7} & 1.617 \cdot 10^{-6} \end{bmatrix}$$

And the correlation matrix was calculated to be:

$$R = \begin{bmatrix} \frac{\sigma^2_O}{\sigma^2_O} & \frac{\sigma^2_{OH}}{\sigma_O \sigma_H} \\ \frac{\sigma^2_{OH}}{\sigma_O \sigma_H} & \frac{\sigma^2_H}{\sigma^2_H} \end{bmatrix} = \begin{bmatrix} 1 & 5.367 \cdot 10^{-2} \\ 5.367 \cdot 10^{-2} & 1 \end{bmatrix}$$

It was tested, via a test for correlation (t-test), that the correlations between the measurement errors of the two data sets were insignificant at test level 5%. Thus, the measurement error covariance matrix V can finally be determined to be:

$$V = \begin{bmatrix} \sigma^2_O & \sigma^2_{OH} \\ \sigma^2_{OH} & \sigma^2_H \end{bmatrix} = \begin{bmatrix} 1.385 \cdot 10^{-5} & 0 \\ 0 & 1.617 \cdot 10^{-6} \end{bmatrix}$$

The variance of r_O data is about 10 times higher than the variance on Hp data, which will be important to consider to obtain reliable parameter estimation results.

6.4.4 *Errors-in-Variables Problems*

One of the assumptions used in nearly all parameter estimation approaches is that the independent variable (typically time, but it can be another experimental setting

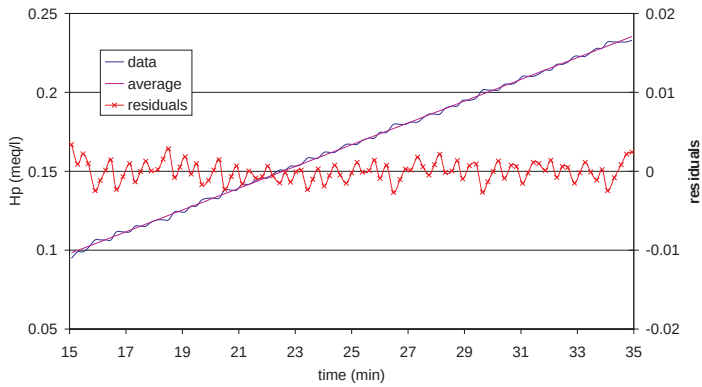

FIG. 6.7. Estimation of measurement variance on Hp data [193] [195].

such as the substrate concentration) for which measurements are made, is free of error. However, it may be that this assumption does not hold.

Occurrence of errors-in-variables problems. When the independent variable itself is also a measurement, e.g. when it represents an experimental condition, the independent variable is not error-free. It is noteworthy that even the time measurement may contain an error if insufficient attention is given to its assessment!

Another frequent origin of errors in the independent variable is due to a transformation of the model, often made to facilitate parameter estimation (i.e. to transform it into a linear regression form), e.g. the Eadie & Hofstee transformation of the Monod function:

$$\mu = -K_S \frac{\mu}{S} + \mu_{max}$$

where, since μ contains measurement error, the independent variable (μ/S) in this simple linear regression is not error free (it may be that the original independent variable S can be considered error free as it is an experimental setting).

The more popular Lineweaver-Burk linearisation of the Monod-function

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \cdot \frac{1}{S} + \frac{1}{\mu_{max}}$$

on the other hand, is not violating this assumption since the independent variable (1/S) in this transformed equation is still free of error. We have seen above, however, that this transformation has other deficiencies and should be looked at carefully too when parameters are to be estimated with it.

Errors in the independent variable may go up to 10% of the errors in the dependent variable without major effect on parameter estimates, i.e. the assumption of error free independent variables is then sufficiently met [218].

Parameter estimation in an errors-in-variables setting. The question we address here is what we should do in case we have to deal with an errors-in-variables problem. Basically, the parameter estimation problem just becomes a special nonlinear estimation problem, even for a model that is linear in the parameters [218]. In the case of a least squares objective function (see below), two kinds of residuals must be considered simultaneously and adjoined in a single residual vector. Calling Y_k the (vector of) dependent variables and X_k the (vector of) independent variables at time t_k, the residual vector becomes:

$$\varepsilon_k(\theta) = \begin{bmatrix} Y_k - \hat{Y}_k(\theta) \\ X_k - \hat{X}_k(\theta) \end{bmatrix} \tag{6.34}$$

With this residuals vector the least squares problem can be addressed if the covariance matrix V_k between these residuals is known entirely, i.e. the objective function to minimise becomes

$$J(\theta) = \sum_{k=1}^{N} \varepsilon_k^T(\theta)\, V_k^{-1} \varepsilon_k(\theta) \tag{6.35}$$

It is assumed in this development that the predicted values do not deviate too much from the real values.

The example below will illustrate how the problem can be tackled even in case a correlation exists between the "dependent" and "independent" variables.

Example of an errors-in-variables problem: Interpretation of a respirogram. As mentioned above, errors-in-variables problems may be created (sometimes unintentionally) when a parameter estimation problem is reformulated in a supposedly easier form. This was purposefully done to facilitate the introduction of the estimation of biokinetic parameters from respirograms in Kong *et al*. [149]. We repeat the example here.

Plotting a typical OUR_{ex} versus time data set typically results in a profile as in Figure 6.8. This oxygen uptake rate curve contains the similar information as the Monod growth curve in defining the relationship between growth rate μ and substrate concentration S.

The substrate degradation rate r_s and S are related to the measured OUR_{ex} in the following way (see also Section 3.2):

$$\begin{aligned} r_S &= \frac{OUR_{ex}}{1-Y} \\ S(t) &= \frac{1}{1-Y}\left(\int_0^{t_{fin}} OUR_{ex}(t)\,dt - \int_0^{t} OUR_{ex}(t)\,dt\right) \end{aligned} \tag{6.36}$$

Where: Y = yield coefficient (mg COD biomass/mg COD substrate consumed)

t_{fin} = time at which OUR_{ex} returns to zero (min.)

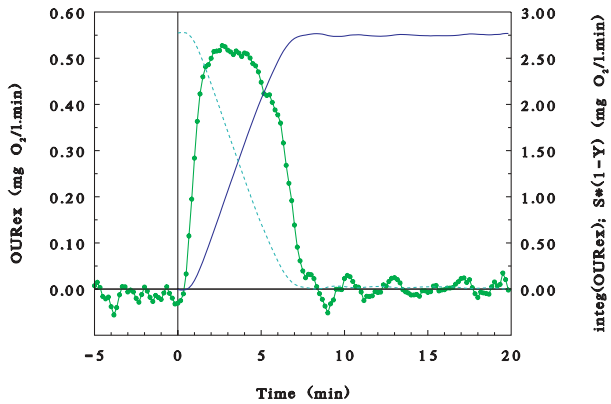

FIG. 6.8. OUR data collected in a respirometer (symbols); integral of the respiration rate, i.e. the consumed oxygen (up-curve) and remaining substrate in the reactor (down-curve).

r_s = substrate degradation rate (mg O_2/l. min)

For illustrative purposes, the cumulative OUR_{ex} and corresponding substrate concentrations in function of time have been combined in Figure 6.8.

Kong *et al.* [149] illustrate how the well-known Monod hyperbolic curve appears when one plots the "growth rate" $(1\text{-}Y)*r_s$ as a function of the "substrate concentration" $(1\text{-}Y)*S$ (Figure 6.9).

This curve is similar to the one obtained from continuous culture experiments in which growth rates are measured for different substrate concentrations. Note, however, that the substrate concentration in such chemostat experiments is measured very accurately and that the independent variable can therefore be considered to be essentially free of error. Hence, standard nonlinear parameter estimation can be applied to estimate the biokinetic parameters.

However, the assumption of absence of error in the substrate concentrations plotted in Figure 6.9 certainly does not hold. Indeed, it is calculated from the original OUR_{ex} data that contain considerable measurement error (see Figure 6.8).

Spanjers and Keesman [236] tackled this errors-in-variables problem in the proper way, i.e. they established the objective function (6.35) with residuals vector:

$$\varepsilon_k(\theta) = \begin{bmatrix} r_k - \hat{r}_k(\theta) \\ S_k - \hat{S}_k(\theta) \end{bmatrix}$$

where, for notational convenience, r_k is the respiration rate (i.e. *OURex* in Figures 6.8) and S_k the short term BOD (i.e. $(1\text{-}Y)S$ in Figures 6.8 and 6.9) at time t_k.

The covariance matrix V could be determined quite nicely because the functional relationship between the "dependent" and the "independent" variable can be

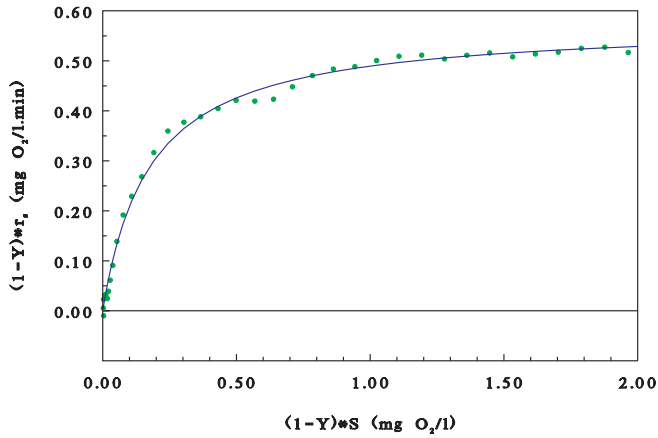

FIG. 6.9. Plot of the respiration rate versus the remaining substrate in the reactor as given in Figure 6.8.

formulated (by applying the trapezium integration rule to equation (6.36)):

$$S_k = \frac{1}{2}\sum_{i=k}^{N-1}\frac{r_{i+1}+r_i}{t_{i+1}-t_i}$$

The expectation of S_k for constant sampling interval and integration interval h then follows from:

$$E[S_k] = E\left[\frac{1}{2}\sum_{i=k}^{N-1}\frac{r_{i+1}+r_i}{h}\right] = \frac{1}{2h}\sum_{i=k}^{N-1}(\bar{r}_{i+1}+\bar{r}_i)$$

where E is the expectation operator and $\bar{r}$ indicates expected respiration rates. Also, the variance of S_k at each time instant can be expressed in terms of the characteristics of the measurement error ξ_k of r_k, i.e. an average of zero and a constant variance λ,

$$var[S_k] = var\left[\frac{h}{2}\sum_{i=k}^{N-1}(r_{i+1}+r_i)\right]$$

$$= \frac{h^2}{4}var\,(r_k + 2r_{k+1} + 2r_{k+2} + \ldots + 2r_{N-1} + r_N)$$

$$\cong h^2\,(N-k)\,\lambda$$

where it is assumed that $var[S_N]=h^{2\lambda}$. Consequently, for the covariance between S_k and r_k we can derive

$$var[S_k, r_k] = E[(r_k - E[r_k])\,(S_k - E[S_k])]$$

$$= E\left[\quad (\xi_k) \qquad \left(\frac{h}{2} \sum_{i=k}^{N-1} (r_{i+1} + r_i) - \frac{h}{2} \sum_{i=k}^{N-1} (\bar{r}_{i+1} + \bar{r}_i) \right) \right]$$

$$= \frac{h}{2} E\left[\quad (\xi_k) \qquad (\xi_k + 2\xi_{k+1} + 2\xi_{k+2} + \ldots + 2\xi_{N-1} + \xi_N) \right]$$

$$\cong \frac{h}{2} \lambda$$

This is also the covariance between the error in S_k and the measurement error in r_k. Hence, the measurement error covariance matrix is written as:

$$V_k = \lambda \begin{bmatrix} h^2 (N-k) & \frac{h}{2} \\ \frac{h}{2} & 1 \end{bmatrix}$$

6.5 Objectives in Parameter Estimation: Estimators

In order to objectify the estimation of parameters (subjective "guessing" parameters by visual inspection of model predictions and data, is still often applied in practice), functions have to be defined that represent the wish to fit a model to the data. These functions are conventionally arranged such that small values represent close agreement between model and data. The model parameters are then adjusted to achieve a minimum in these functions, yielding best-fit parameters. Consequently, this adjustment process is a minimisation problem in many dimensions (i.e. as many as there are parameters to be estimated).

These functions are termed loss, merit, cost or objective function. The choice of such function is indeed one of the first problems to be solved when model parameters are to be estimated. The best known objective function for parameter estimation is the sum of squared errors function

$$J(\theta) = \sum_{i=1}^{N} \left(y_i - \hat{y}_i(\theta) \right)^2 \tag{6.37}$$

where y_i are the observations (in total N observations are available) and $\hat{y}_i(\theta)$ are the model predictions for a given parameter set θ. The objective function J represents all of the information contained in the observations that is not explained by fitting the model to the data [218].

Although this objective function is very well known, its origin is less known to the users. Basically all classic objective functions we will introduce below originate from maximum likelihood (ML) estimators and can be seen as simplifications of the maximum likelihood objective function under given assumptions.

6.5.1 *Maximum Likelihood Estimation*

The starting point in maximum likelihood estimation is the following [203]. If we consider the possible values that parameters can have, we have the intuitive feeling that some values are more likely than others for which the model predictions

look nothing like the data. The problem, however, is how to quantify that intuition. The approach taken in ML estimation starts from the fact that the experimental data are a random sample drawn from the universe of data sets. The question can therefore be asked what the probability is that this data set could have occurred given a particular set of parameters (assuming the model is correct). If the probability of occurrence for a certain parameter set is very small, it can be concluded that the parameters under consideration are unlikely to be correct. From this, the probability of the data given the parameters is identified as the likelihood of the parameters given the data. This identification is based entirely on intuition and has no mathematical basis. If we accept this, then it is, from this point on, only a small step to propose ML estimation as the procedure in which parameters get the values that maximise the likelihood of occurrence of the observations.

Of course, this more philosophical discussion must be concretised in operational functions. Below we will introduce the likelihood functions in case the measurement errors are assumed independent and normally distributed, either homoscedastic (constant variance) or heteroscedastic (non-constant variance). We will see that maximising the resulting likelihood functions leads to reasonably simple objective functions.

6.5.2 *Weighted Least Squares (WLS) or χ^2 Estimation*

When the assumption is made that the measurement errors are independent (uncorrelated) and originating from normal distributions, the likelihood function can easily be constructed as the product of these normal distributions [213]

$$L\left(\bar{y}\,|\theta\right) = \prod_{i=1}^{N} \frac{1}{\sqrt{2\pi}} \frac{1}{\sigma_i} exp\left(-\frac{1}{2}\sum_{i=1}^{N}\left(\frac{y_i - \hat{y}_i\left(\theta\right)}{\sigma_i}\right)^2\right) \tag{6.38}$$

in which $\bar{y}$ is the set of N observations, σ_i is the (estimated) standard deviation of the measurements y_i. For a given data set $\bar{y}$ the maximum likelihood estimates $\theta(\bar{y})$ of the parameters are those values for which the above equation has its maximum. This is equivalent to finding the minimum of the function (due to the negative sign)

$$J\left(\theta\right) = \chi^2\left(\theta\right) = \sum_{i=1}^{N} \frac{1}{\sigma_i^2}\left(y_i - \hat{y}_i\left(\theta\right)\right)^2 \tag{6.39}$$

since all other terms are composed of constants. Minimising this objective function is termed weighted least squares – for obvious reasons – and the estimates are termed weighted least squares estimates.

Alternatively this minimisation is also termed chi-squared fitting. The origin of the latter term warrants some explanation [203]. The χ^2 statistic is a sum of N squares of normally distributed quantities, each normalised to unit variance. However, because we have used the above equation 6.39 to estimate the best parameter

set, the terms in the sum are no longer statistically independent. Still, the probability density function for different values of χ^2 can be derived analytically at its minimum, and is the χ^2-distribution with *N-p* degrees of freedom (with *p* the number of estimated parameters). Comparing the computed χ^2 with tabulated values of the χ^2-distribution for *N-p* degrees of freedom gives a quantitative measure of the goodness-of-fit of the model. If the result fails the statistical test, it means either that

- the residuals are unlikely due to chance fluctuations or, more probably, that
- the model is wrong, or that
- the measurement standard deviations σ_i were underestimated, or that
- the measurement errors were not normally distributed (however, luckily, the test is not very sensitive to this type of deviation).

It may also occur that the χ^2 test is too good to be true. This problem is not likely to be due to a non-normal distribution of the measurement errors, but frequently is caused by an overestimation of the measurement standard deviations. As a rule of thumb it can be stated that a good fit yields a typical value of χ^2 equal to the number of degrees of freedom. Asymptotically, the statistic χ^2 becomes normally distributed with mean *N-p* and variance *2(N-p)*.

In case the measurement errors cannot be estimated, weights can be assigned according to engineering judgement [23]

$$J(\theta) = \sum_{i=1}^{N} w_i (y_i - \hat{y}_i(\theta))^2 \tag{6.40}$$

For instance, one may have the insight that the errors are proportional to the measured value (for instance from an understanding of the measurement principle) [68]. It is then appropriate to use the squared of the inverse of the measured value $(1/y_i)^2$ as the weight w_i. In case transformation of variables is performed (see section 6.3), the weights may often be deduced analytically from the measurement error distributions of the original data [218].

6.5.3 *Ordinary Least Squares (OLS) Estimation*

In case the standard deviations of the measurements are (assumed to be) constant (homoscedastic), the common factor σ_i in equation 6.39 can be dropped from the sum, leading to the objective function

$$J(\theta) = \sum_{i=1}^{N} (y_i - \hat{y}_i(\theta))^2 \tag{6.41}$$

to be minimised to yield the (ordinary) least squares estimates.

6.5.4 *Bayesian Estimation*

As mentioned above, maximum likelihood estimation assumes the parameters to be constants whereas the data are considered to be sampled from a universe of data sets. Bayesian estimation takes this a step further and treats both parameters and measurements as random variables. Bayesian estimation basically updates prior knowledge by considering experimental evidence [23], [213]. To obtain the conditional probability density function (PDF) of occurrence of the parameter set θ given the measurement set $\bar{y}$, Bayes' rule

$$p\,(\theta\,|\bar{y}\,) = \frac{p\,(\bar{y}\,|\theta\,)}{p\,(\bar{y})}\,p\,(\theta)$$

is applied to the a priori PDF of the parameters *p(θ)* and measurements *p(ȳ)* and the conditional PDF that a data set $\bar{y}$ occurs given the parameters θ. The posterior PDF as a function of θ is thus proportional to the likelihood function *p(ȳ,θ)* multiplied by the prior PDF. The three probability density functions on the right hand side have to be specified by the user on the basis of prior knowledge (and the collected experimental data) which makes this approach quite demanding.

It is important to note that this result is not giving a parameter estimate, but rather the complete distribution of the parameter values for the given experimental data set and prior knowledge. If a particular parameter estimate is required for further work with the model, the posterior distribution can be analysed in various ways to yield a parameter point estimate. For instance, taking the mode of the posterior distribution results in the maximum a posteriori (MAP) estimator [19].

It is also noteworthy that if the data do not contain any information on a parameter, this will mean that the posterior PDF of that parameter will not be updated and will remain the same as the prior PDF. This is an important result in case an experiment is non-informative. The parameter non-identifiability that would occur in classic least squares estimation does not cause major problems in obtaining a Bayesian estimate of the parameters [218], [214].

6.5.5 *Robust Estimation*

Problems in nonlinear regression with ordinary or weighted least squares are due to three phenomena:

1. In contrast to what is often assumed, the residual errors are not necessarily normally distributed;
2. In order to weight the residual errors properly, it is necessary that the variance of the error at each measurement point is known. This requires the availability of repeat measurements which are often not made. Extrapolation from a restricted number of repeat measurements (and modelling the evolution of the variance along the time series) may be adequate but may also lead to incorrect weighting, leading to biased estimates;

3. Data points that do not belong to the distribution type to which the majority of the data adhere, so-called outliers, may occur and lead to inaccurate parameter estimates and biased confidence regions.

Rousseeuw and Leroy [221] provide a good introduction into the extensive field of robust estimation and specifically focus on how to detect and deal with outliers.

The robust non-parametric method (i.e. not relying on an assumption concerning a distribution of the errors) applied by Atkins [9] for the estimation of biodegradation model parameters is used here as an illustration of these approaches. It works as follows: For a given parameter set of size p, form p equations by taking p data points from the n available data,

$$\begin{aligned} h(\theta, x_1) - y_1 &= 0 \\ h(\theta, x_2) - y_2 &= 0 \\ \vdots \quad - \quad \vdots \quad &= 0 \\ h(\theta, x_p) - y_p &= 0 \end{aligned}$$

Seek a solution for this set of (nonlinear) equations and repeat this process until all possible combinations of p equations from the N data have been used. List and sort the values for each parameter. Take the median value of that sorted list as the estimate for the parameter considered.

It should be noted that this method heavily relies on the adequacy of the solution method for the many sets of (nonlinear) equations. Atkins [9] indeed reports failure of the [45] method he applied in his work and also complains that the amount of computer time needed to obtain an estimate with this non-parametric method could be prohibitive. On the other hand he also illustrates that bias in the estimates due to the inappropriate use of a WLS objective function could be minimised.

Another robust procedure, applied by Hardwick *et al.* (1991), seeks to maximise the number of runs (also termed zero crossings, see Section 3.3.2) of the residuals sequence obtained with a parameter set. Own experience on respirometric data, however, has found that this method is not very successful due to many local minima problems, i.e. the search algorithm gets stuck at a given number of runs and is not progressing further as no gradient in the number of runs objective function can be discerned. Modification of the method by adding a term that reflects the quality of fit gave a slight but not sufficient improvement in the search efficiency.

6.5.6 *Alternative Objective Functions*

Many other, less statistically underpinned objective functions have been applied, all of them reflecting a certain interpretation given by the users of an optimal parameter estimate.

- Absolute deviations (1-norm) (minimise the sum of absolute deviations),
- Min-max objective function (infinite norm) (minimise the maximum absolute error),
- Maximise the number of sign changes in the sequence of residuals. Note the similarity with one of the structure characterisation methods (run test),
- Minimise first lag autocorrelation. Here too, note the link with structure characterisation methods.

6.5.7 *Selecting a Set of Feasible Parameters*

In some approaches the aim of parameter estimation is not to obtain a parameter point estimate but one is content with a set of feasible parameters that result in model predictions with certain properties, for instance, they give predictions for which the maximum deviation from the data points is less than a certain value. In this respect the development and use of the so-called HSY approach by Hornberger and Spear [129] and Young [288], the set-membership method by Keesman and van Straten [143] and the GLUE-methodology by Beven and Binley [28] are noteworthy. Let us go a bit more in detail on the set-membership and GLUE approaches.

Set-membership. In case detailed characterisation of errors is not possible due to a limited length of data records, or if the residuals have non-random components as a result of model inadequacy or systematic measurement errors, a statistical approach will give unreliable results. Under these circumstances, a deterministic error characterisation in terms of lower and upper bounds only will be a good alternative. This reasoning has led to the development of the so-called set-membership methods [174].

Basically, the parameter space is divided into a behaviour and a non-behaviour space, where the former space contains all parameter sets that give rise to model predictions that are completely contained within an error band around the experimental data (called the behaviour set). The MCSM (Monte Carlo Set-Membership) algorithm [141], [144] can handle this division. The key idea is that randomly selected parameter vectors which result in a model response consistent with the behaviour set belong to the feasible set. Note that by choosing an appropriate error bound, the feasible parameter set can be reduced to a singleton and in this way a unique parameter estimate is obtained from this feasible parameter set method.

As an illustration the work done by Vanrolleghem and Keesman [264] is given. The data set given in Figure 6.10 depicts a respiration rate time series collected by an on-line respirometer at a Dutch wastewater treatment plant. The outer full lines delimit the behaviour set corresponding to the data depicted as symbols and a certain error bound. The feasible model output set is given by the two lines closer to the data points. This set contains all simulation results corresponding to

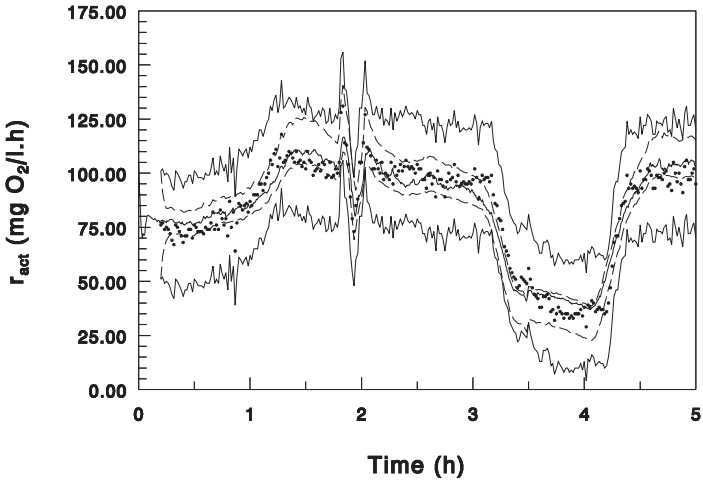

FIG. 6.10. Set-membership method applied to a respiration rate time series collected at a full-scale WWTP: Measurement set (symbols), behaviour set (outer full lines), feasible model output data set (middle dashed lines) and singleton trajectory (centre full line).

parameters whose simulation results were contained completely in the behaviour set. Finally, the single line closest to the data points is the trajectory corresponding with the unique parameter set that is obtained when the error on the measurement set is reduced to such an extent that only a single parameter set is left that gives simulation results that are all contained in the behaviour set.

In another example Vanrolleghem and Keesman [264] also compared the MCSM feasible parameter set with the 95% confidence region of parameters obtained with other parameter estimation methods. The result of a three-parameter estimation problem in which a Monod-model was fitted to a batch respirometric data set, is given in Figure 6.11. It shows that the approximate confidence region is contained completely in the MCSM parameter set, albeit that it is lying on the lower end of the three parameters estimated. Note that the range of the MCSM feasible parameter set is considerably larger than the 95% confidence region. This is of course caused by the large error band that was chosen in this study around the respiration rate data. Theoretically, the size of the feasible parameter set can be reduced to a singleton by reducing this error band adequately.

GLUE. Similarly to the Set-membership approach, the basis of the GLUE approach of Beven and Binley [28] is that any parameter set combination that predicts output variables reasonably well is considered equally likely. It is based upon making a large number of runs of a given model with different sets of parameter values, chosen randomly from specified distributions. Each set of parameters evaluated is assigned a goodness-of-fit value of being the "true" system simulator. The

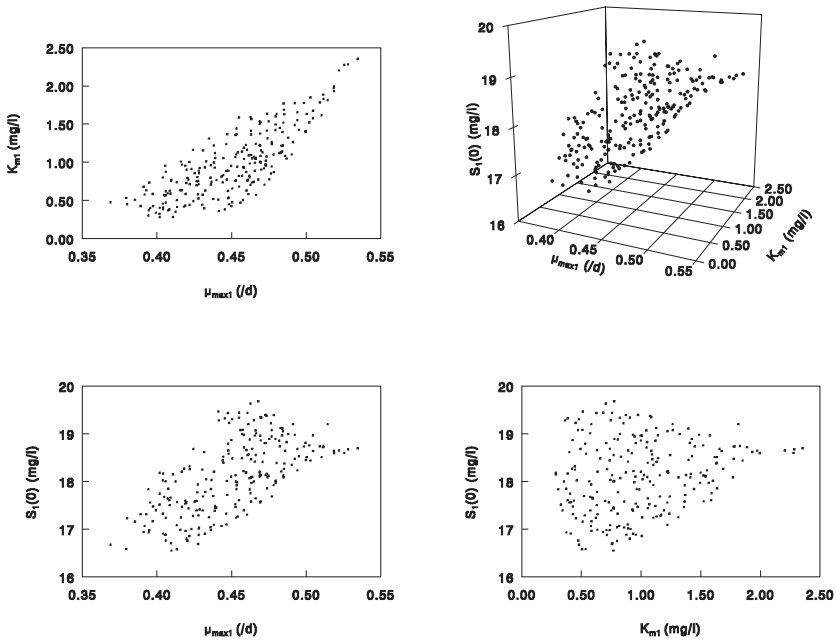

FIG. 6.11. Set-membership parameter estimation of a Monod-model for a set of batch respiration rate data using an error band of 0.1 mg O_2/l.min.

GLUE approach is divided into the following steps:

1. Define a goodness-of-fit function for output data. The choice of function can be crucial to the results of the procedure. Further, a criterion based on the goodness-of-fit function for accepting or rejecting a parameter set must be determined.
2. Define initial ranges or distributions of parameter values to be considered.
3. Sample the parameter space to obtain realisations or simulations of the model. It is most common to use Monte Carlo simulation with uniform parameter distributions.

Going through the steps above yields empirical joint distributions for model parameters. The scatter plots in Figure 6.12 exemplify the approach.

6.5.8 *Multi-Objective Functions*

For some applications, a model must be able to simulate different aspects of the system. For instance, one aim of a model may be to allow a good representation of the average behaviour of the system, whereas the focus of another application may be in the accurate prediction of peak behaviour.

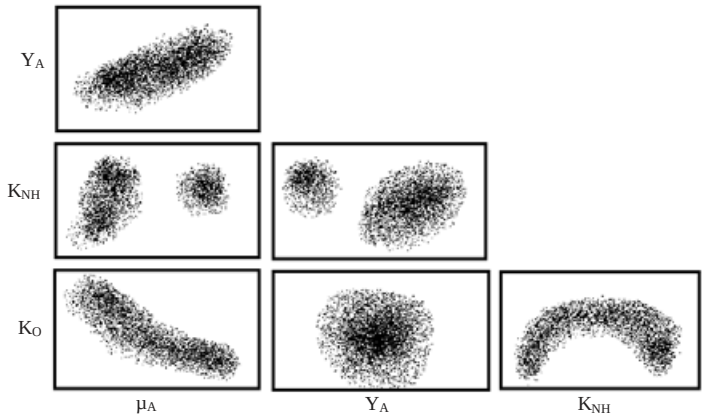

FIG. 6.12. Feasible parameter set obtained by applying the GLUE approach to a nitrification model (4-dimensional) parameter estimation problem.

Many studies have been devoted to find the most adequate objective functions for particular applications. Although in many cases it may be quite straightforward to select an appropriate objective function (see discussion above), no clear-cut answer or selection procedure is currently available. Hence, one is left with the concept that a modeller can choose among different parameter sets obtained with different objective functions that seem equally plausible [235]. It is noteworthy that this multi-objective equivalence of several parameter sets is quite different from the rationale behind the above mentioned set membership, GLUE or HSY approaches in which a single objective can be fulfilled by a range of possible parameter sets.

To deal with the multiple objectives, conveniently written as a set of objective functions

$$J(\theta) = \{J_1(\theta), J_2(\theta), \cdots, J_m(\theta)\}$$

a Pareto set P of solutions is pursued corresponding to various trade-offs among the objectives. This Pareto set is defined such that any member parameter set θ_i has the following properties [235]:

1. For all non-members θ_j at least one member θ_i exists such that the objective $J(\theta_i)$ is strictly less than $J(\theta_j)$. This allows to partition the parameter sets into "good" solutions (Pareto solutions) and "bad" solutions;
2. It is not possible to find θ_j within the Pareto set such that $J(\theta_j)$ is strictly less than $J(\theta_i)$. By "strictly less than" it is meant that $J_k(\theta_j) < J_k(\theta_i)$ for all k=1,...,m. In the absence of additional information, it is not possible to distinguish any of the "good" (Pareto) solutions as being objectively better than any of the other good solutions (i.e. there is no uniquely "best" solution).

The resulting "good" (Pareto) parameter sets therefore are each able to fulfil one of the objectives better than any other member of the Pareto set, but the trade-off will be that some other characteristics of the system's behaviour are less-well described/predicted.

6.5.9 *Multivariate Estimation*

So far all objective functions were only dealing with a single variable for which a number of N observations were available to fit to the model. In practice we of course have many experimental set-ups in which more than one variable is measured, for instance substrate and biomass measurements. It is then quite logical to try to fit a model to each of the outputs it can predict. However, it is also evident that not all of the variables are as trustworthy and that, in view of a certain purpose of the model, it may be more important to predict some variables better than others. To deal with this, it is logical to adopt the Weighted Least Squares (6.39) or ML objective function to express the "optimality" of a parameter set and use the weighting factors to reflect the importance or reliability of the different variables.

Recall that the definition of the Fisher Information Matrix (see Section 5.3) was already developed on the basis of a general WLS objective function written in matrix format:

$$J(\theta) = \sum_{i=1}^{N} \left(y_i\left(\hat{\theta}\right) - y_i \right)^T Q_i \left(y_i\left(\hat{\theta}\right) - y_i \right) \tag{6.42}$$

We recognise the vector of different output variables y_i available for each time instant i and the weights matrix Q_i in which each output variable and each combination of variables is given a weight in the calculation of the objective function value. Note that this weights matrix can be different for each time instant i, for instance to reflect a time-varying quality of the measurements or process conditions. Typically the weights are chosen as the inverse of the measurement error covariance matrix, just as can be seen in the definition of the WLS-objective functional for a single output variable (6.39).

6.5.10 *Multiresponse Estimation*

The above describes how to deal with multiple output variables for which data are available. In some cases multiple experiments are conducted to estimate a single set of parameters. The simplest example is the availability of repeat experiments. However, in some cases it can be advantageous to perform multiple experiments under slightly different conditions but with the aim to estimate a common set of parameters. De heyder *et al*. [68] took advantage of such experiment design to solve an identifiability problem: experiments were conducted at different mass transfer intensities $K_L a$. The fact that multiple experimental data sets are available for the same output variable is known under the term multiple response data

sets. The estimation of parameters with such data sets is called multiresponse estimation. An early example of this is given in Johnson and Berthouex [135]. Mathematically, multiple responses are considered in the same way as different output variables. However, each of the responses is typically weighted equally although one may want to express in the weights that one experiment was more reliable than another.

As an example below a quite general weighted least squares criterion is given in which multivariable (number of variables *Nvar*) data sets are available for *Nresponse* experiments.

$$J(\theta) = \sum_{k=1}^{Nresponse} w_k \sum_{j=1}^{Nvar_k} w_{jk} \sum_{i=1}^{Ndata_{jk}} w_{ijk} \left(y_{ijk}\left(t_{ijk}\right) - \hat{y}_{ijk}\left(\theta, t_{ijk}\right)\right)^2$$

6.6 Minimisation Approach

As mentioned above finding the best parameter estimates typically involves minimising the deviation of the model's predictions from the data points using one of the objective functions J given above. Depending on whether the parameters are linear in the model or nonlinear, the solution methods of this minimisation problem are quite different. For linear parameters a one step calculation gives the best estimates, whereas for nonlinear parameters (as an illustration, see the second introductory example of this chapter) we have to resort to numerical methods that search the parameter space in a systematic way.

6.6.1 *Linear Parameter Estimation*

For problems where the parameters are linear in the model (e.g. linear regression using least squares), the parameter estimates are easily found by differentiating the objective function J with respect to each of the parameters, set these derivatives to 0, and solve the resulting system of equations for the unknown parameters.

In general, a model linear in the parameters can be rewritten in the following form:

$$y_i = \phi_i^T \theta \tag{6.43}$$

where θ is the parameter vector, ϕ_i is termed the regressor, y_i contains the terms that are independent of the parameters and the index i refers to time. The choice of a discrete time representation of the model is natural considering that estimation will be done on the basis of experimental data that are available at sampled time instants.

As an example we will consider a mass balance for a substrate that takes part in two conversions: a growth reaction (with a first order dependency of the conversion rate to the substrate concentration) and a maintenance reaction:

$$\frac{dS}{dt} = DS_{in} - DS - \frac{1}{Y}\alpha SX - m_S X \tag{6.44}$$