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Identification and modelling of aerobic hydrolysis – application of optimal experimental design

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Abstract: Hydrolysis mechanism plays a dominant role in the delicate balance of electron donor/electron acceptor ratios in BNR and EBPR systems as an important carbon source. In this study, the surface-saturation-type hydrolysis kinetics was investigated based on respirometric measurements, within the context of the theoretical and the practical identifiability of mathematical models. The identifiable parameters of a selected model were derived from respirograms. In addition, the information from the experiments was evaluated on the basis of Optimal Experimental Design (OED) methodology for different initial conditions of the batch respirometric experiment.

Keywords: modelling; hydrolysis kinetics; theoretical identifiability; practical identifiability; respirometry; Optimal Experimental Design (OED)

NOTATION

b_H	Endogenous decay rate for heterotrophs (day^{-1})
C_S	Biodegradable COD conc ($S_S + X_S$) ($mgCOD\,dm^{-3}$)
f_E	Inert fraction of endogenous biomass (dimensionless)
FIM	Fisher Information Matrix
k_h	Maximum hydrolysis rate (day^{-1})
K_S	Half saturation constant for heterotrophic growth ($mgCOD\,dm^{-3}$)
K_x	Half saturation constant for hydrolysis ($COD\ cellCOD^{-1}$)
$ML(V)SS$	Mixed liquor (volatile) suspended solid conc ($mgSS\,dm^{-3}$)
S_{sini}	Initial readily biodegradable COD conc ($mgCOD\,dm^{-3}$)
X_{sini}	Initial slowly biodegradable COD conc ($mgCOD\,dm^{-3}$)
Y_H	Heterotrophic yield coefficient ($cellCOD\ COD^{-1}$)
X_{Ha}	Heterotrophic active biomass ($mgcellCOD\,dm^{-3}$)
$\hat{\mu}_H$	Maximum heterotrophic growth rate (day^{-1})

INTRODUCTION

Hydrolysis is an important process which initiates the degradation of slowly biodegradable substrate in wastewaters. This process is an integral part of activated sludge models for the kinetic description of the utilization of the slowly biodegradable substrate. It is, by nature, defined as a process slower than heterotrophic growth and, this way, it usually becomes the rate-limiting step for the biodegradation of organic carbon. The slowly biodegradable fraction, X_S, represents the bulk of the biodegradable substrate in the majority of wastewaters.[1] As a relatively slow process, hydrolysis is generally the decisive step for the quality of the biological effluent. This statement is especially true for industrial wastewaters with more complex substrate compositions.[2,3] Hydrolysis also plays a dominant role in the delicate balance of electron donor/electron acceptor ratio in BNR and EBPR systems.[4–6] Appropriate design, control, and upgrade

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of wastewater treatment plants therefore necessitates accurate and rapid information gathering on the degradation kinetics and quantity of substrate available.

The quantity and kinetic information for substrate present in the wastewater can rapidly be collected by laboratory-scale batch tests with an appropriate mixture of wastewater and biomass.[7–10] In the literature, many batch and semi-continuous respirometric methods have been proposed together with alternative hydrolysis rate equations[8,9,11–14] but still difficulties remain with respect to the appropriate modelling concept. The difficulties for the accurate and representative characterization of wastewater and biomass are not only based upon the modelling pitfalls but also upon the microbiological aspects. From the modelling point of view, the accurate identifiability of hydrolysis kinetics depends on the complexity and structure of the model and on the experimental conditions.

A large number of parameters makes the activated sludge models difficult to identify and may result in parameter correlation, leading to significant uncertainties in the model. One solution is to assume that some of the parameters – difficult to determine from the experiment – have to be fixed to default values. The assessed biokinetic parameters can be used as default values which favour the estimation of the remaining unknown model parameters.[15] The second solution is to break up the complete model into submodels. However, the batch experiments designed for sub-model identification must be sufficiently informative for the parameter subsets under study. The information obtained from the experiment and an improvement in the accuracy of the estimation of model parameters can be evaluated with the application of Optimal Experimental Design methodology.[16,17]

In batch tests, the initial substrate to biomass, S_0/X_0, ratio plays a crucial role in the accurate assessment of the parameters related to microbiological and modelling aspects. If the ratio of S_0/X_0 is very large, significant changes can occur in the culture during the assay, resulting in parameters that are not reflective of the culture as it existed in the environment from which it was removed.[9] From a microbiological point of view, the adaptation period and response of mixed culture population can be observed during the sequential addition of subtrate in kinetic batch tests.[18] The nature of the kinetic experiments is important because some of them may alter the history of the activated sludge culture and cause biased characterization of biomass.[9,19,20] The reason is that activated sludge is composed of a population of microorganisms that each interact differently to biodegradable components. Through competition, the sludge composition may alter and therefore the history of the experiment is important. In the literature, different substrates are classified according to their different degradation rates and, accordingly, the biodegradation kinetics of slowly biodegradable substrate in activated sludge systems is represented by the hydrolysis mechanism. In activated sludge models, the hydrolysis mechanism is commonly described by means of a surface-saturation-type of reaction. This equation can be expressed as:

$$k_h \frac{X_S/X_H}{K_X + X_S/X_H} X_H \text{ or } k_h \frac{X_S}{K_X X_H + X_S} X_H \quad (1)$$

where

k_h: maximum hydrolysis rate (d^{-1})
K_X: half saturation constant for hydrolysis (mgCOD/mgCOD^{-1})
X_S: slowly biodegradable COD (mgCOD/dm^{-3})
X_H: heterotrophic active biomass (mgCOD/dm^{-3})

In this approach, the rate of hydrolysis depends upon the magnitude of two kinetic coefficients, namely, the maximum hydrolysis rate, k_h, and the half saturation constant for hydrolysis, K_X. Numerical values of these coefficients are wastewater specific and exhibit a significant variation, especially for industrial wastewaters.[21,22] The generally adopted procedure for the experimental assessment of k_h and K_X under aerobic conditions involves model evaluation and curve fitting of respirograms. There are extensive data presented in the literature based on this procedure, for different wastewaters. A careful evaluation of the procedure reveals that it is more likely to generate, not a single set, but a relatively large number of different coefficient pairs equally applicable to the experimental data. The calibration of the model can be carried out successfully even using a domain of parameter values to be estimated.

In this context, the scope of the study was first to define a systematic approach for the determination of the most appropriate coefficients that can be extracted from respirograms within the framework of a surface-saturation-type of hydrolysis kinetics. In the second step, the proposed approach was experimentally tested on textile effluent, a typical wastewater with a significant slowly biodegradable substrate fraction. It also covered the accurate estimation of the hydrolysis coefficients for two different sets of initial experimental conditions. This step also included the comparative evaluation of the information obtained from the experiments through the Fisher Information Matrix (FIM) for the two runs.

MATERIALS AND METHODS

Model and wastewater selection

Multi-component activated sludge models[23–27] are becoming more popular, since the degradation kinetics and fate of different substrates can be easily interpreted by means of experimental observations and modelling studies. In this study, a commonly used surface-saturation-type hydrolysis mechanism was investigated for an industrial wastewater. Textile wastewater was selected as a case study since it

Process	Parameters				
	S_S	X_S	X_H	S_0	Rate
Growth	$-1/Y_H$		1	$-(1-Y_H)/Y_H$	$\hat{\mu}_H \frac{S_S}{K_S+S_S} X_H$
Hydrolysis	+1	−1			$k_h \frac{X_S/X_H}{X_S+X_S/X_H} X_H$
Decay			−1	$(1-f_E)$	$b_H X_H$
Parameter, ML^{-3}	COD	COD	Cell COD	O_2	

Table 1. Matrix representation of activated sludge model

$Y_H = 0.67$ (cellCOD COD^{-1}),[27] $b_H = 0.19\ day^{-1}$.

contains high amounts of slowly biodegradable substrate.[3,22,28] This feature is advantageous for the characterization of the kinetics of slowly biodegradable substrate in the framework of the selected simple model presented in Table 1.

The state and parameter vectors of the selected model are shown in Table 1 where $\tilde{S} = [S_0, S_S, X_S, X_H]$ and $\tilde{p} = [k_h, K_X, \hat{\mu}_H, K_S, Y_H, f_E]$ respectively. The heterotrophic yield coefficient, Y_H, was assumed to be known and the inert fraction of endogenous biomass, f_E, was accepted as 0.2 in all similar activated sludge models. The endogenous decay rate, b_H, was determined with the method proposed by Ekama *et al.* [10]

Theoretical identifiability

The investigation of identifiable parameters of a selected model has an important role in view of reliable unique parameter estimations. There are few available methods applicable to non-linear models to derive identifiable parameter subsets.[29–32] Since the application to a non-linear model is relatively simpler than the other methods, the Taylor Series Expansion method was used here to study the theoretical identifiability.[32] Other researchers have also applied this method to activated sludge models.[33–36] The Maple V (Waterloo) software package was used for the Taylor series expansion of the hydrolysis model.

The model was investigated through two different modelling approaches together with the Taylor series expansion. In the first approach, it was assumed that there was no growth of X_H during the duration of the experiment. The expansion of the hydrolysis model showed that the parameter subsets for the growth and the hydrolysis processes could be estimated simultaneously under the assumption that no change in X_H took place. For this case, six parameter subsets can be derived from the respirogram (Table 2, columns 1 and 3). In the second approach, the equation for the growth of X_H was additionally included in the series expansion (eqn (2)). Thus, the maximum hydrolysis constant, k_h, could be separated from the parameter subsets (Table 2, column 4).

Maple V is one of the most advanced symbolic manipulation programs available for addressing the structural identifiability evaluation using the Taylor Series Expansion. It has been applied successfully in previous studies. However, the computing limitations of the program did not allow the complex model to be coined when biomass growth was included in the model. These limitations have also been observed before.[16,33,36,37] However, *a pseudo steady state approximation*[16] for the growth process was made since it became too complex for Maple V to solve the growth and hydrolysis processes together. The change in the S_S concentration was assumed to be zero, as shown in eqn (3). According to the model, the growth process is limited by the hydrolysis rate. It should be noted here that the parameters for the growth and the hydrolysis could not be found simultaneously, because the solution of the series expansion became too complex to solve, as mentioned above.

$$\frac{dX_H}{dt} = \hat{\mu}_H \frac{S_S}{K_S + S_S} X_H \quad (2)$$

$$\frac{dS_S}{dt} = 0 \quad 0 < t < t_{end} \quad (3)$$

According to the Taylor series expansion method, two different parameter subsets can be found for the hydrolysis kinetics, depending on whether the biomass

Growth kinetics[36]		Hydrolysis kinetics (this study)	
No biomass growth	Biomass growth	No biomass growth	Biomass growth[a]
(1)	(2)	(3)	(4)
$(1-Y_H)\frac{\hat{\mu}_H X_{H0}}{Y_H}$	$(1-Y_H)\frac{X_{H0}}{Y_H}$	$(1-Y_H)k_h X_{H0}$	$(1-Y_H)X_{H0}$
$(1-Y_H)S_{S0}$	$(1-Y_H)S_{S0}$	$(1-Y_H)K_X X_{H0}$	$(1-Y_H)K_X X_{H0}$
$(1-Y_H)K_S$	$(1-Y_H)K_S$	$(1-Y_H)X_{S0}$	$(1-Y_H)X_{S0}$
–	$\hat{\mu}_H$	–	k_h

Table 2. Identifiable parameter combinations for hydrolysis model

[a] Based on the pseudo-steady state approximation.

growth during the experiment is considered or not. The identifiable parameter subsets that can be found by parameter estimation are listed in Table 2. This means that the parameter combinations give unique values, but the individual parameters of that combination can vary as long as the value of the combination is maintained. As a result, the hydrolysable COD fraction, X_{S0}, the maximum hydrolysis rate, k_h, the half saturation constant for hydrolysis, K_X, and the initial active heterotrophic biomass, X_{H0}, are found to be structurally identifiable if the heterotrophic yield coefficient, Y_H, is known.

Practical identifiability

In a theoretical identifiability study the data, in this case OUR data are assumed to be noise-free. In reality, however, this situation is not achievable in practice due to the lack of precision of equipment and environmental conditions.[18] Moreover, problems such as finding highly correlated parameters may arise if insufficient and/or highly noise-corrupted data are collected from real laboratory experiments. For instance, the Monod-type growth model is a good example when the maximum heterotrophic growth rate, $\hat{\mu}_H$, and the half saturation constant for growth, K_S, are found to be highly correlated if the experiments are poorly designed.[38] Batch experiments were used to keep the study simple, the only experimental degree of freedom used was the initial biomass concentration. Also, previous work in this field of OED applied to activated sludge systems[11,17,34] only focused on this kind of problem. In this way, we could compare the results. It is clear that much more complicated experimental design problems (substrate dosage during the experiment, sampling times, temperature changes, oxygen concentration) can be tackled but these are beyond the scope of this paper. So, the information obtained from the experiments can be augmented either by setting better initial conditions or applying different experimental techniques (eg fed batch operation, multiple additions of substrate) which makes the experiment much more informative for reliable and accurate parameter estimation.[17,33,37–42] In addition, the mathematical techniques used for the estimation of the model parameters may have numerical problems such as slow convergence to the solution or getting stuck in a local minimum.[16] The Fisher Information Matrix (FIM) is regarded as a cornerstone of OED methodology.[17] In general, the FIM summarizes the sensitivity functions for output variables and measurement errors with respect to quantification, especially the shape of the joint confidence regions of parameters.[41,43] The FIM can be expressed by the formula given in eqn (4):

$$\mathrm{FIM} = \sum_{i=1}^{N} Y(t_i, p)^T Q_i Y(t_i, p) \qquad (4)$$

where $Y(t_i, p)$ is the output sensitivity function with respect to the parameters. The weighting matrix, Q_i is typically the inverse of the measurement error covariance matrix. There are several scalar functions that can be derived from the FIM in order to compare the information obtained from the experiments which have, for instance, different initial conditions. Within the scope of this study, two of these scalar functions will be examined. The first one is the D-Criterion which is calculated as the determinant of the FIM known to be inversely proportional to the volume of confidence region: the higher the value of determinant, the smaller the confidence region. The second one is the Mod-E criterion that deals with the shape of the confidence region. It corresponds to the condition number of the FIM. The condition number is the ratio of the largest eigenvalue over the smallest eigenvalue. If the minimum eigenvalue of FIM is found to be zero, the information content of the experiment becomes zero. This happens, for instance, when there is a strong dependency or high correlation between the estimated parameters, and means that the condition number approaches infinity. The WEST++ software package was used for the simulation and calculation of the sensitivity trajectories for the estimated parameters.

Experimental setup

In this study, textile wastewater was sampled from a textile mill with a COD value of 1200 mg dm^{-3}. Biomass was acclimated to the textile wastewater before conducting the respirometric batch experiments. Two different batch experiments were carried out under different initial conditions with different dilutions and S_0/X_0 ratios as proposed by Eleama *et al.*[10] A nitrification inhibitor (Formula 2533™, Hach Co) was added to suppress the oxygen utilization due to nitrification. The S_0/X_0 ratio was adjusted to 0.16 and 0.09 gCOD gVSS^{-1} for the first and second experiments, respectively. The oxygen uptake data were measured with a Manotherm RA-1000 continuous type respirometer with a sampling rate of sample per minute.[44] COD and Mixed Liquor Volatile Suspended Solid (MLVSS) measurements were carried out according to standard methods.[45] The initial conditions of the experiments are summarized in Table 3.

Table 3. Initial conditions for experiments 1 and 2

Condition	*Unit*	*Experiment 1*	*Experiment 2*
S_0/X_0 ratio	gCOD gML VSS^{-1}	0.16	0.09
Total biomass, X_{MLVSS}	mgVSS dm^{-3}	1530	1400
Total volume, V_T	dm^{-3}	2.700	2.230
Wastewater volume, V_{ww}	dm^{-3}	0.500	0.230

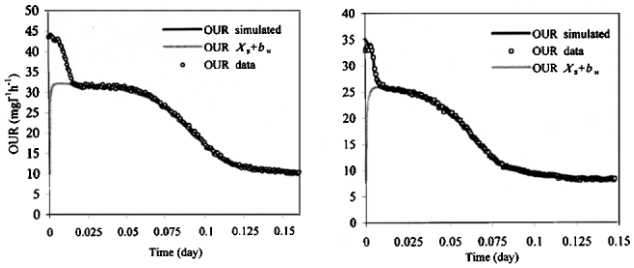

Figure 1. Simulated and measured OUR values, experiment 1 (left), experiment 2 (right).

RESULTS AND DISCUSSION

Parameter estimation and sensitivity analysis

Parameter estimation study using the OUR profiles was carried out for the initial values of the following state variables: the initial readily biodegradable COD, S_{S0}, slowly hydrolysable COD, X_{S0}, and the initial active heterotrophic biomass, X_{H0}, and kinetic parameters: the maximum hydrolysis rate, k_h, the half saturation for hydrolysis, K_X, the maximum heterotrophic growth rate, $\hat{\mu}_H$, and the half saturation constant for heterotrophic growth, K_S. In order to test model validity, the parameter estimation study was conducted in two stages. In the first stage, the parameter estimation was carried out for the experiments individually. However, in the second step, multi-experimental fit[16] was applied using two sets of OUR data. These two different estimation methods show that the selected model is reproducible because individual and multi-experimental parameter estimation gave identical parameter values. As shown in Fig 1, good simulation fits on real OUR profiles are attained after estimation of the parameters. It is even difficult to differentiate the simulation from the experimentally-obtained data.

As illustrated in Fig 1 by the simulated OUR profile that corresponds to the slowly biodegradable COD, the period in which the hydrolysis process is rate-limiting is clearly observed. Figure 1, also shows that the degradation of the readily biodegradable COD, S_S, was terminated after 0.02 and 0.01 days for experiments 1 and 2, respectively. Evidently, the first OUR experiment, started with a much higher initial S_0/X_0 ratio (0.16), reached the endogenous level later than experiment 2.

Based on the selected model, it is also clear from the respirograms that a longer second plateau was obtained at high S_0/X_0 ratios, which can be interpreted as a saturation of the aerobic hydrolysis depending upon the X_S concentration in the reactor. Hence, the second shoulder of the respirogram contains information on the hydrolysis kinetics. Estimated parameters and calculated absolute standard deviations are given in Tables 4 and 5. According to the results, the individual and the multi-experimental estimations give nearly identical results, showing good simulated fits on the experimental respirograms. It can be concluded from Tables 4 and 5 that the relative error for K_S has

Table 4. Estimated initial state variables and parameters and deduced sludge and wastewater characteristics

	Experiment 1		Experiment 2	
Parameter	Value	STD[a]	Value	STD[a]
$\hat{\mu}_H$ (day^{-1})	1.088	0.015	1.081	0.027
K_S (mgCOD dm^{-3})	0.46	0.08	0.65	0.11
k_h (day^{-1})	1.140	0.005	1.146	0.009
K_X (COD cellCOD^{-1})	0.0102	0.0003	0.0107	0.0004
S_{sini} (mgCOD dm^{-3})	9.84	0.13	5.00	0.09
X_{sini} (mgCOD dm^{-3})	137.01	0.31	77.20	0.26
X_{Ha} (mgcellCOD dm^{-3})	1585	3.7	1367	4.6
S_{Sww} (mgCOD dm^{-3})[b]	53		49	
X_{Sww} (mgCOD dm^{-3})[b]	740		748	
C_{Sww} (mgCOD dm^{-3})[b]	793		797	
X_T (VSS) – Experimental	1530		1400	
X_{Ha}/X_T (cell-COD)	0.72		0.69	

[a] Standard Deviation.
[b] Concentration in wastewater.

comparably higher values than that of the other parameters.[17,37,41] The errors contributed by each estimated parameter were also checked by the eigenvalue decomposition of the covariance matrix. The most uncertainties pertaining to the parameters of K_S, K_X and $\hat{\mu}_H$ are found to be important (results not shown). For the first experiment, the sensitivity analysis was carried out using the absolute-relative sensitivity function. This function measures the absolute change in OUR for a 100% change in the parameter. The sensitivity trajectories for the estimated parameters are illustrated in Fig 1. The sensitivity trajectories for the growth process (Fig 2, left) show that the effects of $\hat{\mu}_H$, K_S and S_{S0} are negligible after the first plateau (compare Fig 1, left). The excitation of the sensitivities are confined in the first OUR plateau.

Figure 2 reveals that the shapes of the sensitivity trajectories obtained for $\hat{\mu}_H$ and K_S are quite similar but follow opposite directions with time. Since the magnitude of the sensitivity for K_S is lower than that of

Table 5. Estimated initial states and parameters (multi-experimental fit)

	Multi-experimental fit	
Parameter	Value	STD[a]
$\hat{\mu}_H$ (day^{-1})	1.104	0.011
K_S (mgCOD dm^{-3})	0.509	0.049
k_h (day^{-1})	1.168	0.011
K_X (COD cellCOD^{-1})	0.0106	0.0002
S_{sini1} (mgCOD dm^{-3})	9.97	0.117
S_{sini2} (mgCOD dm^{-3})	4.55	0.15
X_{sini1} (mgCOD dm^{-3})	139.73	0.59
X_{sini1} (mgCOD dm^{-3})	76.39	0.38
X_{Ha1} (mgCOD dm^{-3})	1571	8
X_{Ha2} (mgCOD dm^{-3})	1317	8

[a] Standard Deviation.
Subscripts 1 and 2 refer to experiment number.

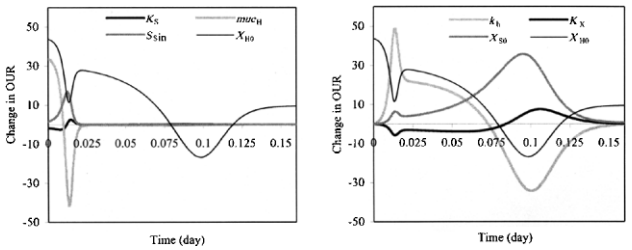

Figure 2. Absolute-relative (AR) sensitivity trajectories of the parameters for growth (left) and hydrolysis (right) (experiment 1).

$\hat{\mu}_H$, the uncertainty pertaining to K_S is higher. This conclusion can also be derived from the results of parameter estimation. As discussed above, the relative standard deviation is calculated to be around 16% for K_S, which is approximately 10 times larger than for the other parameters. However, the situation is different for the hydrolysis process. The parameters of k_h, K_X and X_{S0} have influences on the OUR profile during the whole of the experiment. The sensitivity of k_h shows that the effect of this parameter reaches its maximum during the transition between OUR plateau (the first and second drops in OUR). The sensitivity trajectory for S_{S0} is only visible during the first plateau. As expected, after the first plateau the sensitivity of S_{S0} is zero since the hydrolysis process is then the rate-limiting step according to the simulation. However, the sensitivity profile of the initial slowly biodegradable COD component, X_{S0}, is active until the endogenous level is reached. Maximum sensitivities for X_{S0} were obtained when the OUR was between the hydrolysis and the endogenous levels, indicating that this provides important information on this parameter.

The sensitivity trajectories presumably look similar for K_X and k_h, possibly leading to a correlation between these parameters. Indeed, an increase in one parameter can be compensated by the increase in the other parameter. The contour plots of the sum of squared errors (SSE) with respect to the different parameters exert a valley-like shape of the confidence region, illustrating the extent of this correlation (Fig 3). The greater sensitivity for the slowly biodegradable COD fraction, X_{S0}, is located between the second and the third OUR plateau (compare Fig 1, left). This period contains much more information for the estimation of the X_{S0} fraction.

However, it should be noted here that some difficulties may arise for the accurate estimation of this fraction if the hydrolysis plateau cannot easily be distinguished from the endogenous level. This situation is quite common, especially for respirometric measurements carried out for domestic wastewater under low initial S_0/X_0 (substrate/biomass) ratios.[2,11,15] Therefore, the initial experimental condition should be optimized in order to gain more information both on the X_{S0} fraction and the kinetic constants via OED techniques.[17] As expected, the parameters for the hydrolysis kinetics are not effective during the endogenous level and at the beginning ($t = 0$) of the OUR experiment because the sensitivities are almost zero. The contours of the sum of squared errors (SSE) concerning the hydrolysis constants, k_h, K_X and the initial heterotrophic active biomass, X_{H0}, are plotted two by two against each other, as illustrated in Fig 3 in a two-coordinate system. These figures give information about the uncertainties of the parameters relative to each other. For instance, it can be concluded from Fig 3 that the estimation of X_{H0} is much more accurate than that of K_X. The optimum values of the parameters (ie their estimates) are located in the middle of the inner circle. From a modelling point of view, the parameter ranges are also quite small both for X_{H0} and K_X. However, the shape of the confidence region is valley-like. On the contrary, the k_h and K_X parameters are inversely proportional to each other according to Fig 4. The shapes of the contour plots are circle-like which is a desirable situation for accurate parameter estimation. However, it should be noted that one should consider the overall effect of the estimated parameters in a multi-dimensional vector space (with dimension = number of parameter).

OED concept

In this section, two experimental runs are evaluated on the basis of the OED concept using the scalar functions of the Fisher Information Matrix (FIM). For these batch experiments, only the initial biomass concentration in the reactor is considered as a degree of freedom. However, absolute values of this component cannot be determined without calibration. Still, the added mixed liquor and the calibrated active biomass are proportional. In this respect, the effect of different initial active heterotrophic biomass concen-

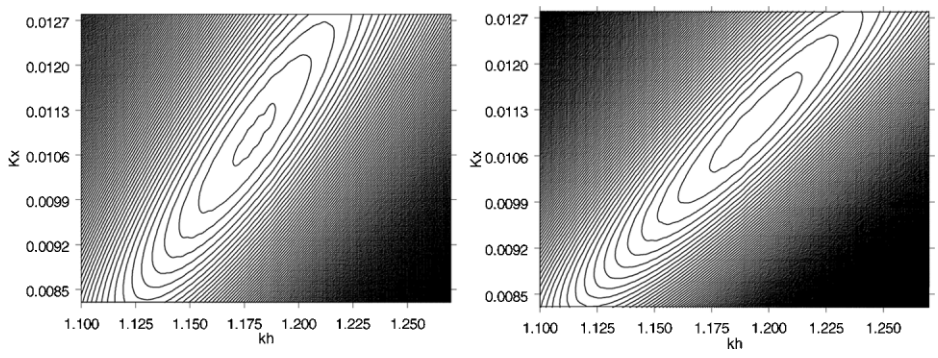

Figure 3. SSE contour plots for k_h and K_x, experiment 1 (left), experiment 2 (right).

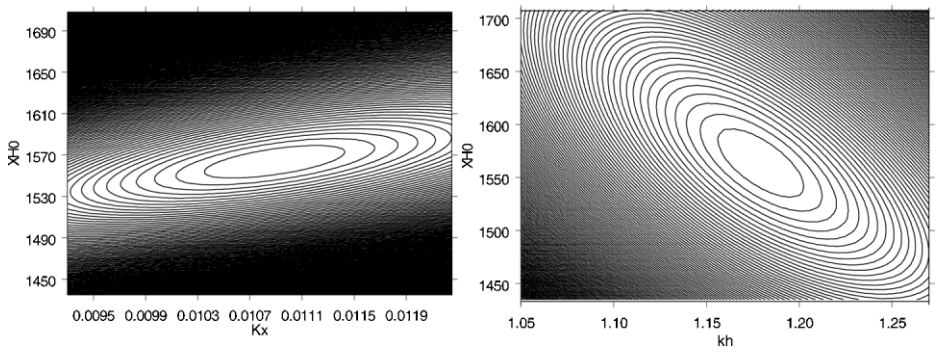

Figure 4. SSE contour plots for K_X and X_{H0} (left) and k_h and X_{H0} (right) for experiment 1.

trations on the information contents can be evaluated by means of the D-criterion and the Modified-E criterion (Fig 5). According to the Mod-E criterion, the experiments were found to be optimal with an initial active biomass concentration around 1500 mg dm^{-3}, ie the parameter correlations are minimal. Increasing the active biomass concentration results in an increase of the Mod-E criterion value. As a result, the degree of parameter correlation is higher. In other words, the shape of the confidence ellipsoids becomes more elongated with the increase in the initial value of X_{H0}. The optimality of the experiments could not be satisfied for the D-Criterion (the determinant of FIM) at 1500 mg dm^{-3} initial active biomass, ie the volume of the multi-dimensional confidence ellipsoid is getting smaller as the initial active biomass is increased. According to Fig 5, starting up the experiment with a much higher initial biomass will provide overall smaller confidence regions but larger parameter correlations will result. In short, by lowering the S_0/X_0 ratio, the parameters can be estimated more accurately, but they become more correlated.

It should be stressed here that the first OUR plateau which is important for the growth kinetics ($\hat{\mu}_H$, K_S, X_{H0}) can be non-informative due to the sudden (too short) peak in the first plateau under very low S_0/X_0 ratios.[15,46] The multi-experimental fits can be suggested for the experiments performed under high and low S_0/X_0 ratios for accurate and unbiased estimation of the parameters for growth and hydrolysis kinetics, because a shift in the value of one parameter may inevitably influence the values of the other parameters. On the other hand, the confidence regions only for k_h and K_X were found to be comparably smaller and elongated for lower S_0/X_0 ratios.[47] If these two experiments are compared with respect to their information contents, it can be seen from Fig 5 that the first experiment contains comparably more information than the second one.

The OED-based methodology applied in this paper is shown to be useful for evaluating the accuracy of parameter estimation for surface-saturation-type hydrolysis kinetics. According to the applied method, besides readily and slowly biodegradable COD fractions, the kinetic constants for hydrolysis can be estimated from a batch respirogram. More accurate estimation of parameters could be provided by the full application of Optimal Experimental Design (OED). Since the optimal initial conditions of the experiment are dependent on the parameter values and wastewater type, experimental design should be applied for each case. Generalized conclusions on parameter values may lead to erroneous and biased parameter estimation of hydrolysis parameters. Therefore, hydrolysis parameters should be estimated for each case.

Evaluation of two sets of parallel experiments

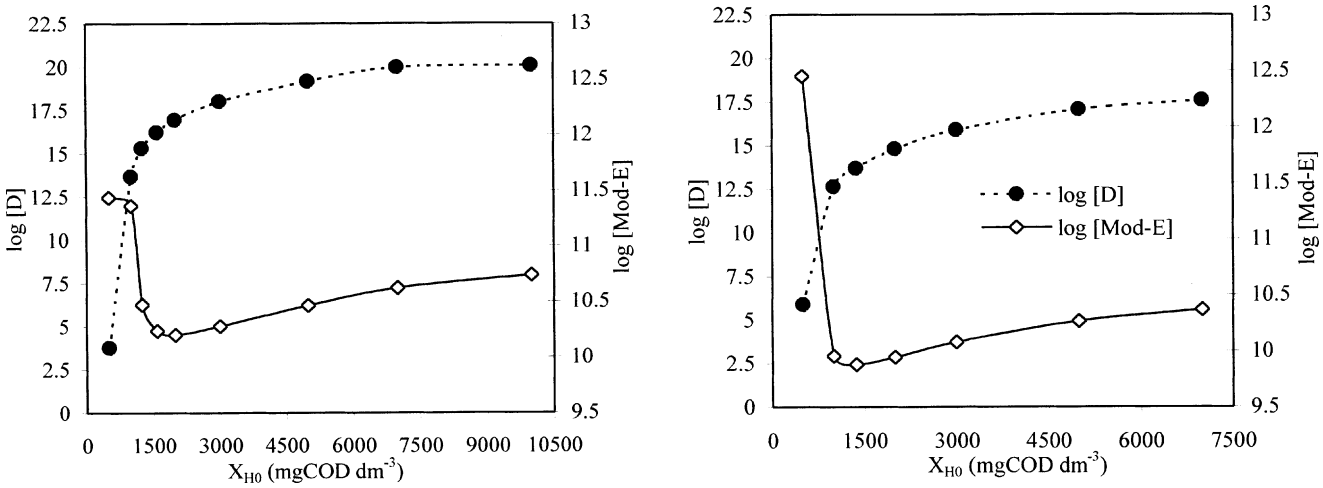

Figure 5. Information obtained from experiment 1 (left) and experiment 2 (right).

carried out at markedly different initial ratios of wastewater and sludge provides a refinement to the procedure, without however, providing, a definitive solution to the problem. It is therefore essential to define an experimental evaluation system that would enable identification of the most appropriate set of coefficients associated with the hydrolysis kinetics.

CONCLUSIONS

The proposed method allows estimation of the kinetic constants and initial states of the surface-saturation-type hydrolysis model. The information content of the experiment can be improved by starting the respirometric experiment under a low S_0/X_0 ratio. Although the confidence region becomes smaller by adjusting the initial conditions to a low S_0/X_0 ratio, the parameter correlations become greater with the increase in initial heterotrophic biomass concentration.

Accurate estimation and model validation can be performed using the multi-experimental fit approach. It also provides a solution for the correlated parameters and high standard deviations which are known to be common problems for the estimation of parameters from batch experiments. As a result, the initial conditions of the experiments should be well defined, depending upon the selected model.

The parameters of the growth and the hydrolysis processes can be estimated quite easily from the batch respirogram if two plateaux are observable. However, the hydrolysis parameters and the concentration of the initial slowly biodegradable COD fraction can only be estimated with the aid of the second plateau and the endogenous OUR levels. In this study, a textile wastewater was tested as an example and for this wastewater, the growth, hydrolysis and endogenous phases can easily be distinguished from each other.

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