Respirometric assessment of biodegradation characteristics of the scientific pitfalls of wastewaters

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Abstract This paper provides an overview of common problems encountered when using oxygen uptake rate (OUR) measurements for the assessment of wastewater characteristics and process kinetics. Emphasis is placed upon pitfalls that would lead to significant errors. It covers model dependency of the OUR measurements and the need to select appropriate models; interpretation of OUR perturbations as a way to identify new model components and processes; the need for simultaneous observation of relevant model components and multicomponent modelling for appropriate evaluation of OUR measurements; parameter identifiability problems and the effect of active biomass concentration and the endogenous decay rate on model simulation and calibration. Relevant experimental OUR data from previous studies are presented to illustrate and underline common scientific pitfalls.

Keywords Activated sludge models; COD fractions; experimental design; industrial wastewaters; oxygen uptake rate; process stoichiometry and kinetics; respirometry

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Introduction

The recognition of dissolved oxygen as a significant model component and its incorporation into mechanistic models is perhaps a key milestone in the basic understanding and interpretation of complex biochemical reactions taking place in the activated sludge process. Together with nitrate, dissolved oxygen is one of the two important final electron acceptors in biological processes of practical significance for organic carbon and nutrient removal from wastewaters. Consequently, it is instrumental in setting the electron balance between biodegradable COD, biomass and electron acceptor for aerobic processes, which will be the focus of this paper. In recent activated sludge models, emphasis has been mainly placed upon the rate of utilisation of dissolved oxygen, conveniently defined as the oxygen uptake rate (OUR), which is an overall process rate reflecting the cumulative impact of all oxygen/energy consuming reactions.

With modern instrumental technology, OUR profile obtained for a wastewater under selected operating conditions is readily measurable as a dynamic response in batch reactors. With the pioneering work of Ekama *et al.* (1986), the OUR profile is introduced as a useful instrument for the assessment of biodegradable COD fractions and model parameters. Related principles and procedures have been incorporated first into ASM1 (Henze *et al.*, 1987), and then into the models that followed ASM1. Then, a wide spectrum of different applications have been developed to improve the experimental assessment of wastewater characterization and process kinetics (Sollfrank and Gujer, 1991; Kappeler and Gujer, 1992; Spanjers and Vanrolleghem, 1995; Avcıoğlu *et al.*, 1998; Çokgor *et al.*, 1998; Sözen *et al.*, 1998; Karahan-Gül *et al.*, 2002a; Insel *et al.*, 2003).

The convenience of using OUR and developing new procedures has perhaps encouraged researchers to oversee some of the common problems and errors that may possibly affect the accuracy and reliability of the evaluations. The OUR profile is merely a result

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of the sequence of biochemical reactions in the system and scientific feedback is necessary on the nature of these reactions before any evaluation can be made. Moreover, it reflects the combined effect of a number of parameters, which cannot always be accurately determined beforehand. The lack of necessary information may lead to a distorted image for all the estimations. This paper gives an overview of significant issues that need to be taken into consideration for the respirometric assessment of biodegradation, based on available state-of-the-art experience.

Respirometric assessment of biodegradation

Model dependency – selection of the appropriate model

Respirometry becomes a useful tool only as an integral component of an appropriate model. Every modelling approach has to rely upon a correct assessment of the available biodegradable substrate. Thus, the preliminary assessment mechanism for modelling should be able to differentiate inert/residual organic components. An OUR profile, when properly generated, may be used to yield the magnitude of the total biodegradable substrate. In fact, the OUR area above the endogenous respiration level directly gives the amount of oxygen consumed at the expense of all available substrate. This amount is proportional to total biodegradable COD, C_S:

$$\Delta O_2 = C_S(1 - Y_H)$$

where, Y_H is the heterotrophic yield coefficient. Therefore, C_S may be computed provided that Y_H is known. Orhon and Okutman (2003) define a way to assess soluble and particulate inert COD components, S_I and X_I, by a sequence of OUR measurements on raw and filtered (soluble) substrate followed by simple mass balance. The level of these components may also be verified by direct measurements (Orhon *et al.*, 1999a). Figure 1 illustrates the calculation of C_S for domestic sewage, using a Y_H value of 0.67 mg cell COD/mg COD previously computed for the same wastewater (Orhon *et al.*, 2002; Orhon and Okutman, 2003).

Conversely, with a known/predetermined amount of substrate, the OUR curve can be used for the assessment of a yield coefficient. At this stage, it is crucially important to identify the relevant mechanism for substrate utilisation and use the appropriate model for OUR evaluation. Obviously, this evaluation is model-dependent and may give a totally misleading result. In fact, depending on experimental design, substrate utilisation may be due to direct microbial growth (Henze *et al.*, 1987) or storage (Krishna and van Loosdrecht, 1999). In the case where direct growth applies, the OUR profile can yield Y_H (Sollfrank and Gujer, 1991; Çokgor *et al.*, 1998). These types of evaluations have been extensively used for domestic sewage and industrial wastewaters (Orhon *et al.*, 1995,

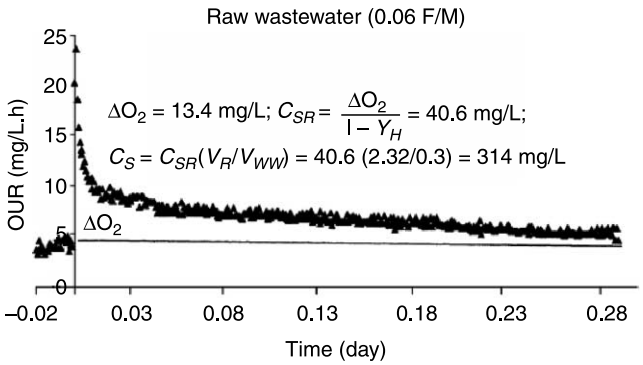

Figure 1 Assessment of C_S for domestic sewage using an OUR profile (Orhon and Okutman, 2003)

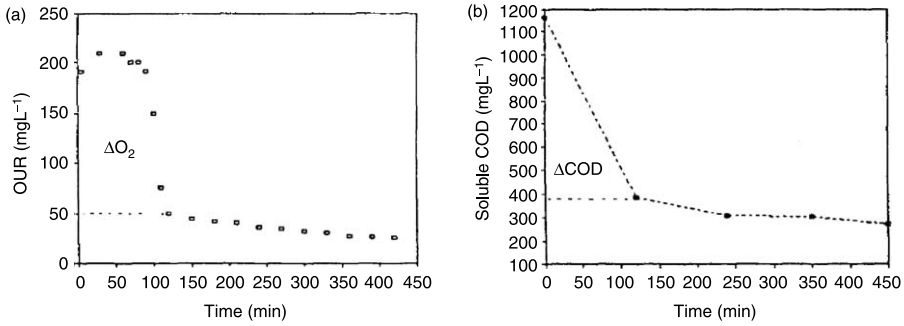

Figure 2 Assesment of Y_H using profiles of (a) OUR and (b) soluble COD for confectionery wastewater for assessment (Orhon *et al.*, 1995)

1999b). A typical example is presented in Figure 2, which shows the OUR profile used for the experimental assessment of Y_H for confectionary wastewaters, yielding a Y_H value of 0.69 mg cell COD/mg COD.

A similar OUR profile may result from the conversion of biodegradable substrate to internal storage material and may be used for the computation of the storage yield, Y_{STO} (Karahan-Gül *et al.*, 2002a). Figure 3 gives two OUR profiles, one for the storage of acetate and the other for a glucose/acetate mixture, giving a Y_{STO} value of 0.78 mg cellCOD/mgCOD for acetate and 0.87 mg cellCOD/mgCOD for glucose (Karahan-Gül *et al.*, 2002b). The same problem also applies to the assessment of the corresponding process rates, i.e. the maximum heterotrophic growth rate, μ_H (Kappeler and Gujer, 1992; Sözen *et al.*, 1998) or the maximum storage rate, k_{STO} (Koch *et al.*, 2000). The value of OUR interpretation obviously depends upon the relevancy of the adopted model for substrate utilisation.

Interpretation of OUR perturbations – new model components

Often, parts of an experimental OUR profile obtained for a given wastewater deviate from the one derived for a selected appropriate model and deviations from the theoretical OUR curve still persist despite calibration efforts with a wide range of model coefficients (Figure 4). These types of experimental perturbations with respect to model calibration should be regarded as positive indication of new model components/processes not defined in the selected model. A typical example of such an evaluation is the differentiation of two groups of slowly biodegradable substrate with a dual hydrolysis model. Figure 5a illustrates the deviation of the model profile from the experimental OUR data for

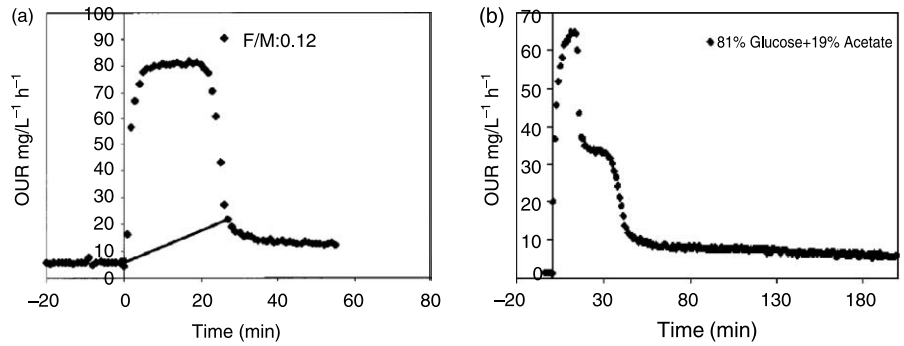

Figure 3 OUR profiles for the storage of (a) acetate and (b) for a glucose/acetate mixture – 81% glucose (Karahan-Gül *et al.*, 2002a, b)

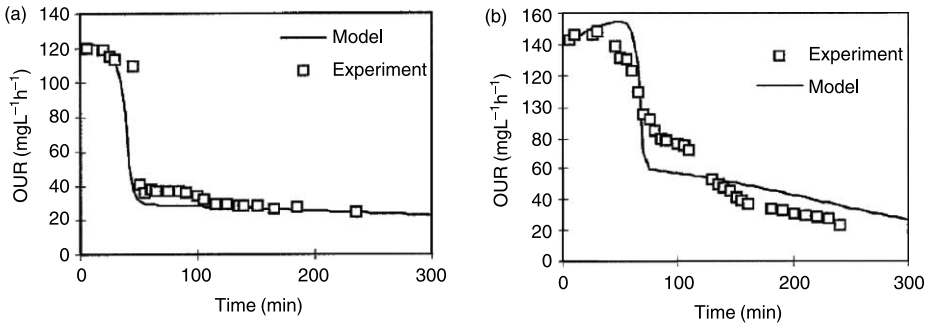

Figure 4 Discrepancy between experimental data and single hydrolysis model for (a) tannery wastewater (b) textile wastewater (Orhon *et al.*, 1998)

domestic sewage when the evaluation is made on the basis of a single hydrolysis mechanism covering the entire slowly biodegradable COD. The same data could be calibrated with a dual hydrolysis model (Figure 5b). Similarly, a very slowly degrading settleable COD component could be identified for domestic sewage, from the evaluation of the lower end of the OUR profile (Orhon *et al.*, 2002).

In batch experiments, after addition of substrate, the OUR sometimes exhibits a lag period until reaching maximum respiration level. This observation was attributed to physico-chemical processes of substrate diffusion into floc and dynamics of dissolved oxygen probe (Vanrolleghem *et al.*, 2004). In order to reduce the discrepancy between degradation model and experimental data, the transient (with first-order model) term was included in the growth process. Otherwise, the estimates of model parameters would be biased.

Simultaneous observation of relevant model components – multicomponent modelling

The OUR parameter, although quite useful, may at times be misleading when the effect of more than one microbial mechanism on its structure is overlooked. Models such as ASM1 or ASM3 give a simplified account of microbial processes for substrate utilisation and only define growth or storage as the dominant mechanism. A more appropriate evaluation should therefore include relevant experimental information on all related components, i.e. substrate consumption, storage formation, if any, and electron acceptor utilisation (OUR). Recently, this approach was illustrated by Karahan *et al.* (2006) in the modelling of starch utilisation, where simultaneous observation and modelling of starch, glycogen as storage material and OUR could reveal a mechanism of adsorption, partial storage and simultaneous direct microbial growth. The model could then be calibrated, as

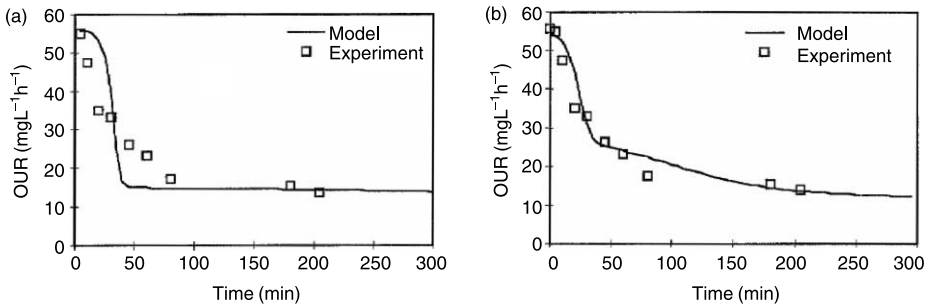

Figure 5 Calibration of the OUR data for domestic sewage using (a) a single hydrolysis model (b) a dual hydrolysis model (Orhon *et al.*, 1999c)

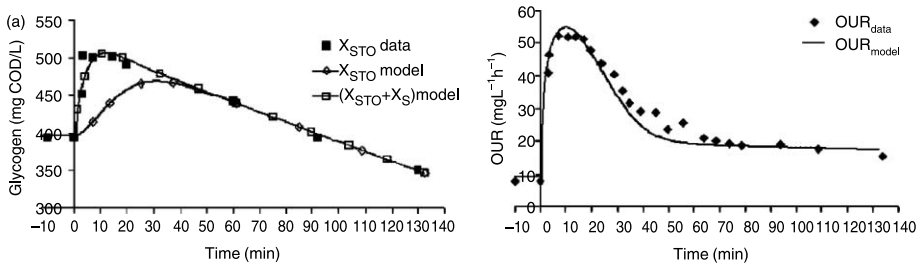

Figure 6 Model calibration of (a) glycogen and (b) OUR, of starch utilisation for storage and primary growth (Karahan *et al.*, 2006)

shown in Figure 6, to yield a more accurate model fit on experimental data using relevant process stoichiometry and kinetics.

Similar results with appropriate model definition and evaluation were also reported for glucose utilisation of *E. coli* under aerobic conditions (Insel *et al.*, 2007). Figure 7a gives, together with glucose utilisation, the OUR data which exhibits quite an unusual profile with two peaks. The first part of this profile, if modelled for microbial growth only, would give a distorted image of growth kinetics. Detailed observation indicated acetate generation and glycogen storage as other mechanisms dictating the shape of the OUR profile, which was then modelled together with acetate and glycogen profiles as shown in Figure 7b.

Experimental design – parameter identifiability

Parameter identifiability of the model is one of the key issues in modelling the degradation with respirometry, which comprises theoretical and practical identifiability steps. Briefly, in theoretical identifiability, the model is evaluated under certain conditions to gather information on extractable parameters. Mathematical techniques are applied on the models (i.e. linearisation, sensitivity analysis) in order to determine parameter subsets under acceptable assumptions (Pohjanpalo, 1978; Walter, 1982). In a practical identifiability step, the parameters (or subsets) are estimated from the model applied on real experimental data (Vanrolleghem *et al.*, 1995; Brouwer *et al.*, 1998; Brun *et al.*, 2002). In practical identifiability, measurement noise and data sampling frequency influence the success of parameter estimation as well. Noisy data make it difficult to get best model fit on data. Hence, the measurement noise should be minimised. The selection of data frequency is also important for calculating the confidence intervals of the estimated

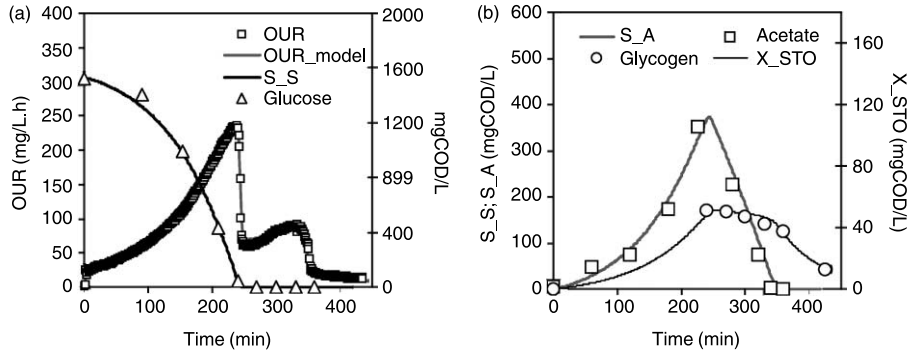

Figure 7 Model simulation of (a) glucose, OUR and (b) acetate and glycogen profiles for glucose utilisation by cultivated *E. coli* (Insel *et al.*, 2007)

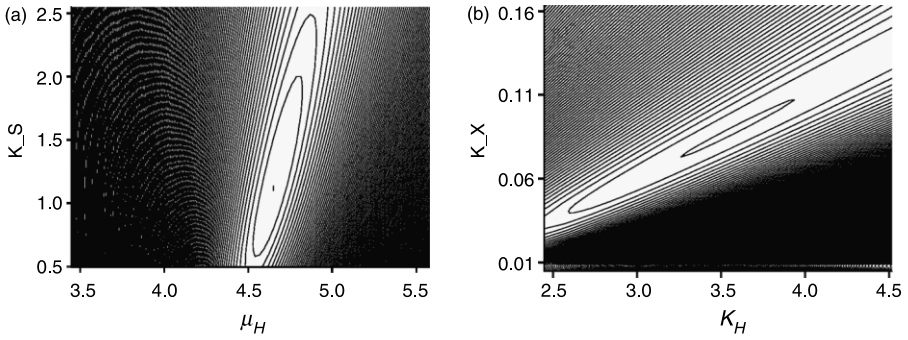

Figure 8 Contour plots of objective function for (a) growth and (b) hydrolysis parameters

parameters. The correlation of measurements results in getting contracted confidence ellipsoids, which does not reflect the real case. The solution is that the measurement should have white noise, governing that there is no autocorrelation between the measurements (Dochain and Vanrolleghem, 2001). In this respect, the optimal measurement frequency should be selected accordingly.

Especially for (semi-) batch experiments, the definition of initial experimental conditions is crucial in the parameter estimation task since it may expose severe identifiability problems such as parameter correlation, non-informative experiments. This may also cause failures in automatic search algorithms (i.e. Simplex, Secant) to find the minimum value of the objective function. Figure 8 illustrates the contour plots of objective function for growth and hydrolysis parameters simulated for the OUR model given in Figure 2b. Although obtaining an excellent model fit, the parameters, especially the hydrolysis process constants, exhibit a high degree of uncertainty exhibiting open contours within the k_h-K_X domain. For instance, more informative experiments were obtained via applying batch OUR experiments under low initial food to microorganism (*So*/*Xo*) ratios for the estimation of growth and hydrolysis parameters. This means that the parameters have narrow confidence intervals under low (*So*/*Xo*) ratios. On the other hand, as a side effect, the parameter correlations increased considerably (Insel *et al.*, 2002, 2003). At this level, optimal experimental design (OED) techniques serve as an excellent tool to remedy the parameter identifiability problems. The (initial) experimental conditions, data frequency can be adjusted via applying OED techniques efficiently (Dochain and Vanrolleghem, 2001; Insel *et al.*, 2003).

Effect of active biomass and endogenous decay rate

In microbial cultures where heterotrophic growth dominates as in most activated sludge systems, the active biomass, X_H, and the endogenous decay coefficient, b_H, are

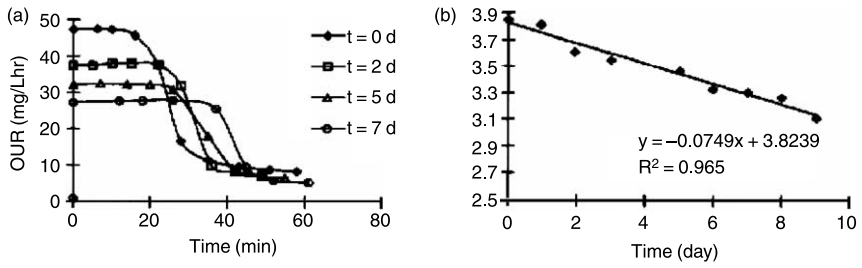

Figure 9 Endogenous decay rate estimation from batch OUR profiles (Avcıoğlu *et al.*, 1998)

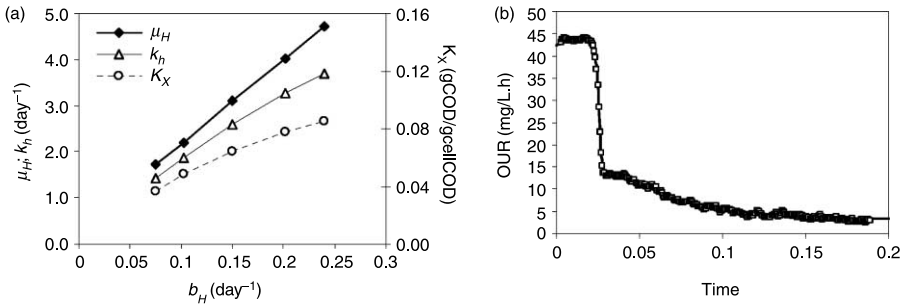

Figure 10 Simulations for (a) b_H effect on estimation of other parameters and (b) calibrated model using OUR profile

parameters with a significant impact on OUR profile, persisting throughout the entire profile. OUR is inherently a function of active biomass (X_H), an important initial model state common to all related parameters, which cannot be identifiable by direct measurements. During the initial period prior to substrate feeding, the OUR level is solely established by $b_H.X_H$, therefore correct information on b_H is imperative for an acceptable estimation of initial X_H (Spanjers and Vanrolleghem, 1995; Insel *et al.*, 2002). The kinetic parameters such as maximum growth rate ($\hat{\mu}_H$) and maximum hydrolysis rate (k_h) were found to be dependent on the active biomass concentration (X_H). Hence, a bias in X_H estimate causes inevitable shifts in the estimates of those kinetic parameters (Dochain and Vanrolleghem, 2001; Insel *et al.*, 2003). Values of b_H in the range of 0.15–0.25 day^{-1} are reported in the literature (Ekama *et al.*, 1986; Sollfrank and Gujer, 1991). This parameter can also be determined independently by long-term respirometric measurements. The procedure developed by Avcıoğlu *et al.* (1998) is interesting in the sense that b_H is estimated independently from X_H concentration. (Figure 1). The procedure is based on batch OUR experiments conducted with endogenous biomass sampled from the main aerated reactor at different time intervals. Thus, as seen in Figure 9, the endogenous decay parameter (b_H) can be extracted with the aid of a decrease in initial OUR levels with time ($T = 0$–7 days). The estimated b_H values for domestic sewage under aerobic conditions were found in the range of 0.08–0.11 day^{-1} (Avcıoğlu *et al.*, 1998).

In the evaluation of the OUR curve, what is conceived as initial X_H depends on the characteristics of the biomass seed used for the respirometric test. Depending on experimental pre-cultivation conditions, the seed may contain a significant amount of particulate slowly biodegradable COD (X_S) and/or storage cell materials (i.e. PHB, glycogen). This interference results in a misleading interpretation of high initial OUR level, due to the combined effect of endogenous decay and biodegradation of remaining substrates, similarly affecting the model calibration procedure and estimation of other parameters. Below, Figure 10 shows the influence of endogenous decay rate (b_H) on the estimation of heterotrophic growth rate ($\hat{\mu}_H$), maximum hydrolysis rate (k_h) and half saturation hydrolysis parameters (K_X). It is clear that shifting b_H value will cause an inverse drift in active heterotrophic biomass (X_H). This latterly affects the estimates of $\hat{\mu}_H$, k_h and K_X, which are directly linked to initial active biomass, X_{H0} (Dochain *et al.*, 1995; Insel *et al.*, 2003).

Conclusions

Respirometry, and especially OUR measurements, has been the most useful experimental instrument today for the elucidation of wastewater character and biodegradation. It serves as an integral component of current multicomponent mechanistic models. It may,

however, become more detrimental than beneficial if not supported by full understanding of relevant microbial mechanisms. Moreover, significant pitfalls have to be expected in the evaluation of OUR profiles if this understanding is not properly translated into modelling. The paper gives detailed account of these pitfalls, namely: model dependency of the OUR measurements and the need to select an appropriate model, the need for simultaneous observation of relevant model components and multicomponent modelling for appropriate evaluation; parameter identifiability problems and the effect of active biomass concentration and the endogenous decay rate on model simulation and calibration of OUR profiles.

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