Important limitations in the modeling of activated sludge: biased calibration of the hydrolysis process

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Abstract The merit of activated sludge models depends on the accuracy and reliability of the information they contain on the wastewater to be treated and the biochemical mechanisms involved. In most advanced calibration studies, respirometry i.e. the measurement of the oxygen utilization rate, (OUR), provides the majority of the required experimental database. However, currently used procedures still involve a number of basic and practical problems. Model evaluation of the OUR data may generate a distorted image of the processes involved. Hydrolysis is the most important, yet the most vulnerable process as far as the experimental assessment of accurate kinetic parameters is concerned. This study intends to provide an overview of major experimental limitations in the modeling of activated sludge, with emphasis on the appropriate experimental design for the assessment of the hydrolysis rate.

Keywords Active biomass; activated sludge; endogenous decay; experimental analysis; hydrolysis; model parameters; model sensitivity; process kinetics; respirometry

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Introduction

Recent developments on the modeling of activated sludge may be regarded as one of the most significant achievements in environmental science and technology today. They provided a giant improvement in the mechanistic understanding of activated sludge. With the support of a few pioneering scientific studies (Dold *et al*., 1980; Ekama and Marais, 1979; van Haandel *et al*., 1981), the introduction of ASM1 brought about a complete revision in the concept of traditional modeling (Henze *et al*., 1987). It was soon modified for endogenous decay and generation of residual metabolic products, a concept quite significant for the biological treatment of industrial wastewaters (Orhon and Artan, 1994). ASM1 was expanded into ASM2 and ASM2d to cover enhanced biological phosphorus removal (Henze *et al*., 1995, 1999). Recently ASM3 was proposed, advocating biochemical storage as the primary mechanism of substrate utilization for organic carbon and nitrogen removal (Gujer *et al*., 2000).

This rapid improvement however, increased the structural complexity of the models, because it is achieved by introducing new concepts that translated as additional *model components* and new *biochemical processes*, each involving a new set of *kinetic* and *stoichiometric parameters*. It should be noted that the merit of the new models mainly depends on the accuracy and reliability of the information they reflect on the wastewater to be treated and the biochemical mechanisms involved. This information must be experimentally determined. It forms the experimental basis of activated sludge modeling. It has to cover (i) wastewater fractions included as model components; (ii) biomass fractions in activated sludge and wastewater, and (iii) various kinetic and stoichiometric parameters defining biochemical processes in the models.

The progress in the modeling of activated sludge covering all aspects of carbon, nitrogen

and phosphorus removal has been so fast that experimental backup lagged behind. It is time now to make the necessary scientific effort to close the gap between modeling and the experimental support it requires. It should be noted that wastewater characterization and experimental evaluation of process kinetics, although an integral part of the comprehensive kinetic evaluation of activated sludge systems, requires a different experience and expertise as compared to modeling. Generally, activated sludge models are regarded as *over-parameterized* and the order of the model should be reduced or simplified with appropriate applications of mathematical techniques. This way, splitting of a complete experimental data set in terms of time segments (Keesman *et al.*, 1997) and/or estimating only the best parameter combinations (Dochain *et al.*, 1995; Weijers and Vanrolleghem, 1997) may be necessary in order to provide correct information on the kinetics. As a result, much more accurate predictions can be derived on the overall performance of an activated sludge process.

The objective of the paper is to highlight selected experimental aspects of activated sludge modeling, mainly for organic carbon removal as it is the major concern for most agro-industries, emphasizing weak points and drawbacks, and this way underlining expected future developments in this area.

Relevance for agro-industries

Biological processes constitute the major treatment step for industrial wastewaters and especially, the effluents from agro-industries. Experimental support for the understanding of biological treatment is more important for industrial wastewaters compared to domestic sewage, mainly because the latter is well studied and defined in terms of commonly accepted parameters, whereas industrial effluents are much more diversified.

Agro-industries generate strong wastewaters with high COD and generally low total N and P. Therefore, the common concern is mainly organic carbon removal and rarely N removal as in the case of leather tanning and meat processing effluents. External phosphorus supply is usually needed as a remedy for nutrient deficiency in biological treatment. Characteristics of a number of selected industrial effluents, together with domestic sewage, are given in Table 1. COD fractionation as shown in the table reveals high residual COD fractions as a common feature of most industrial effluents. Slowly biodegradable COD represents the bulk of their COD content, with magnitudes greatly exceeding that in domestic sewage.

Table 1 Typical characteristics of selected industrial effluents (Orhon *et al.*, 1999a)

Parameters (mgl^{-1})	Agro industries				Domestic
	Tannery	Poultry processing	Textile	Dairy	
Conventional characterization					
Total COD	2,285	2,490	2,400	1,410	430
Soluble COD	1,298	1,770	1,700	1,075	170
TSS	770	418	500	190	350
TKN	160	343	35	63	47
Total P	6	30	34	7	8
COD fractions					
S_{S1}/C_{T1}	0.19	0.11	0.14	0.28	0.10
X_{S1}/C_{T1}	0.60	0.78	0.82	0.64	0.80
S_{I1}/C_{T1}	0.10	0.10	0.14	N*	0.03
X_{I1}/C_{T1}	0.11	0.01	N*	0.08	0.07

* N: negligible

ASM 1 modified for endogenous decay and soluble residual COD generation (Henze *et al.*, 1987; Orhon and Artan, 1994; Orhon *et al.*, 1992) is usually suitable for the modeling of activated sludge for agro-industries. Limited to organic carbon removal for simplicity, it involves, as defined in Table 2, a minimum of 8 model components, 3 processes and a total of 8 kinetic and stoichiometric parameters. The appropriate values of these parameters for industrial wastewaters should be expected to be different as compared to domestic sewage, depending on the processes/chemicals used in each different industrial category. With high slowly biodegradable COD fractions, hydrolysis should be underlined as the important mechanism with the main concern that hydrolysis rates may be significantly lower as compared to domestic sewage, due to organic compounds that are not totally compatible with biological treatment.

Respirometry as a tool for experimental evaluation

Respirometry has been used as a very convenient and useful instrument for providing the experimental information required for the modeling of activated sludge systems (Spanjers and Vanrolleghem, 1995; Spanjers *et al.*, 1998, Vanrolleghem *et al.*, 1999; Petersen *et al.*, 2001). The assessment of inert COD components and related model parameters obviously require other techniques (Germirli Babuna *et al.*, 1991; Orhon *et al.*, 1999b; Petersen *et al.*, 2001) and mass balancing is sufficient for the assessment of the biodegradable COD components, aside from the readily biodegradable COD. A detailed account of wastewater characterization is presented elsewhere (Henze, 1992; Petersen *et al.*, 2001) and it is beyond the scope of this work. Respirometric approaches generally involve observing the electron acceptor utilization profiles in batch reactors initially started with a wastewater/biomass mixture. The oxygen utilization rate (OUR) in an aerated reactor is used for the characterization of aerobic processes and the nitrate utilization rate (NUR) for the computation of correction factors applicable to anoxic conditions.

A suitable substrate to biomass ratio (F/M ratio) needs to be selected for each particular application (Dochain and Vanrolleghem, 2001). Figure 1 shows typical OUR curves that would be obtained for domestic sewage and textile wastewater. Both curves were generated for characteristics generally accepted to describe these wastewaters and an initial heterotrophic active biomass of 200 mg cell COD l^{-1} corresponding to an F/M ratio of 1.35 g COD(g cell COD)$^{-1}$ for domestic sewage and 1.70 g COD(g cell COD)$^{-1}$ for textile wastewater (Table 3). The difference in the shape of the OUR profile is mainly due to a much slower rate of hydrolysis for the textile effluent, requiring a longer period to reach the final endogenous phase, a common pattern for most industrial wastewaters. Accordingly, it would be a mistake to end the experimental observation after a shorter period, as it is necessary to reach the endogenous phase for an accurate evaluation of the OUR test.

Table 2 Matrix representation of an activated sludge model involving residual COD generation

Model Components → **Processes ↓**	S_I	S_P	S_S	X_S	X_H	X_P	X_I	S_O	**Process Rate**
Growth			$-\frac{1}{Y_H}$		1			$-\frac{(1-Y_H)}{Y_H}$	$\hat{\mu}_H \frac{S_S}{K_S + S_S} X_H$
Hydrolysis			1	–1					$k_h \frac{X_S / X_H}{K_X + X_S / X_H} X_H$
Decay		f_{ES}			–1	f_{EX}		$(1–f_{ES}–f_{EX})$	$b_H X_H$
Unit [ML^{-3}]	COD	COD	COD	COD	Cell COD	COD	COD	O_2	

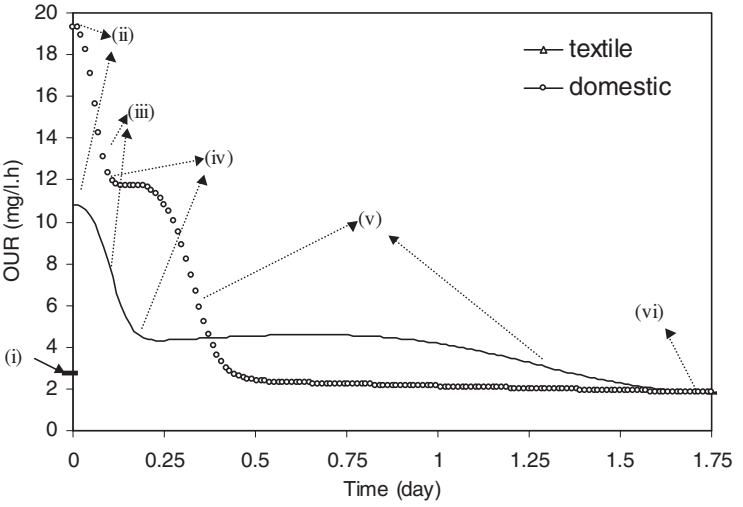

Figure 1 Typical OUR profiles for domestic sewage and textile wastewater

Parts of the OUR curve reflect different levels of sensitivity to the sequence of biological processes in the batch reactor, a valuable asset for kinetic evaluation. Basically, (i) the initial OUR level before substrate addition is determined by the endogenous decay activity, $b_H X_H$; (ii) the starting level of the first plateau is set by the growth activity, $\mu_H X_H$; (iii) the ascending slope of the first plateau, if any, is due biomass growth; (iv) the half saturation constant, K_S is most effective on the slope of the drop after the first plateau; (v) the shape of the OUR curve after the first plateau is mainly governed by the hydrolysis rate, namely, k_h and K_X, and (vi) the last phase reflects again endogenous respiration.

The area under the OUR curve as a whole depends upon the specific biodegradable COD fractions (S_S, S_H, X_S) defining the particular wastewater tested in the respirometric experiment. The relative impact of different kinetic parameters on the OUR curve is best assessed by a sensitivity analysis. Figure 2 illustrates separately the sensitivity analysis for growth and hydrolysis processes carried out for the OUR curve associated with domestic sewage for the half of the experiment shown in Figure 1.

It should be noted here that if the sensitivity trajectories of the parameters are proportional and have the same sign, there is *inverse correlation* between those parameters. On the contrary, if the shapes of the sensitivity trajectories are proportional but with different sign, inevitably, there is a *direct correlation* between these parameters. In this context, the

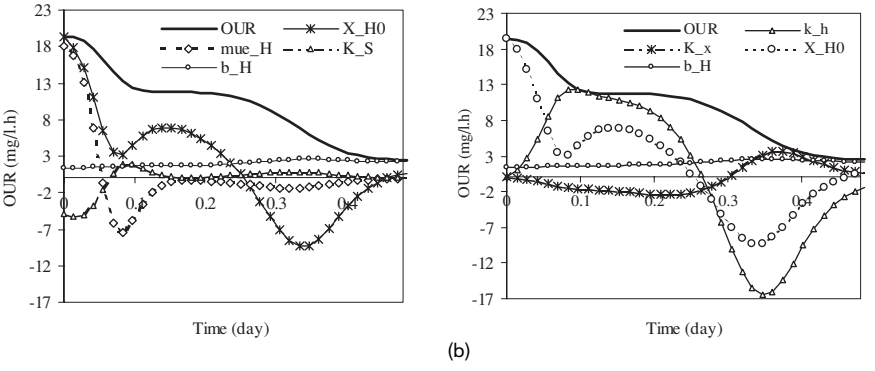

Figure 2 Sensitivity analysis for (a) growth and (b) hydrolysis processes on the OUR curve obtained for domestic sewage [F/M = 1.35 gCOD(g cell COD)$^{-1}$]

figure reflects, as expected, a strong inverse correlation between μ_H and X_H, with the indication that the increase in one parameter can be compensated with a corresponding decrease in the other. The correlation between $\hat{\mu}_H$ and K_S is somewhat different in the sense that the shape of the sensitivity functions is the same but the trajectories follow opposite directions. The figure indicates a similar and strong correlation between the hydrolysis rate parameters k_h and K_X. As will be elaborated on later, this correlation cannot be removed completely for batch tests but may be minimized using Optimal Experimental Design (OED) techniques (Vanrolleghem *et al.*, 1995; Insel *et al.*, 2002; Dochain and Vanrolleghem, 2001).

Experimental evaluation of model parameters

Currently, techniques using respirometry are widely used for the experimental assessment of individual kinetic parameters such as $\hat{\mu}_H$ and b_H. Similar techniques are also defined to asses the active heterotrophic biomass, X_H, a significant model component. Assessment of the hydrolysis parameters k_h and K_X still requires model calibration of the experimental OUR data, provided that necessary information on wastewater characteristics and other kinetic and stoichiometric parameters are previously obtained and included in the evaluation (Vanrolleghem *et al.*, 1999; Petersen *et al.*, 2001).

Experimental assessment of $\hat{\mu}_H$

There are inherent difficulties associated with the traditional methods that use the linearized Monod expression and overall COD and VSS measurements, yielding consistently low $\hat{\mu}_H$ values. New respirometric techniques were developed in accordance with the structure of the new models, based upon the interpretation of the maximum OUR observed during a period with no substrate limitation. Ekama *et al.* (1986) suggested running an aerated batch reactor at a suitably low initial F/M ratio that would generate an observable initial OUR plateau. They defined a basic relationship showing that $\mu_H X_H$ was proportional to the OUR level in this plateau. Another and somewhat tedious procedure was also given to calculate X_H this way leading to the experimental assessment of $\hat{\mu}_H$. It is important to note that the shape of the OUR profile in this experiment is very sensitive to the initial amount of active biomass in the reactor. At the low F/M ratios recommended for the test, it may not be justifiable to neglect the effect of endogenous respiration on the OUR. With this viewpoint, Kappeler and Gujer (1992) developed a similar test initiated with a high F/M ratio, yielding a totally different and exponentially ascending OUR profile. With the assumption that maximum growth can be sustained in the reactor during the period where the readily biodegradable substrate concentration remains sufficiently high, $\hat{\mu}_H$ can be calculated without knowledge of the initial level of X_H in the reactor. The approach used was criticised by Novak *et al.* (1994) with the argument that it may create a microbial growth medium different from conditions associated with continuous-flow activated sludge systems and that during the test fast-growing organisms may be selected giving rise to an overestimation of the maximum growth rate. Grady *et al.* (1996) further stated that conditions before the kinetic set may have an impact on the measured parameters and suggest that such impacts are best prevented when disturbances during the test remain small and the duration of the test is relatively short.

Based on existing experimental information, a $\hat{\mu}_H$ value of around 5–6 d^{-1} is generally adopted for domestic sewage. Results in the same range were also reported for different industrial effluents provided that compounds with inhibitory action were removed prior to the test (Sözen *et al.*, 1998). This observation was explained with the argument that new models related microbial growth only to readily biodegradable substrate, S_S and the nature of S_S, by definition, should not change from one wastewater to the other.

Experimental assessment of b_H

In the traditional modeling approach, the endogenous decay parameter is determined as a lump term, k_d, from the linear relationship between the sludge age and the specific substrate removal rate (Pearson, 1968). The procedure can only define an apparent decay parameter of around $k_d = 0.05\ d^{-1}$ which is lower than the true b_H, value and the underestimation becomes more pronounced as the sludge age increases (Orhon and Artan, 1994). This explains the wide range of k_d values reported in the literature.

The currently developed models, which differentiate between active biomass and inert organic particulate matter, often rely on respirometry for the experimental assessment of b_H. The procedure proposed by Ekama *et al.* (1986) is now widely recognized and routinely used for this purpose. It involves observing the OUR profile obtained from the aerobic digestion of activated sludge with no external substrate over a period of several days. The slope of the plot of ln(OUR) versus time yields the value of b_H. A b_H value of $0.24\ d^{-1}$ at 20°C was reported for domestic sewage and was adopted as a default value in recent models. Similar evaluations for industrial effluents resulted in variable and generally lower b_H values. Spanjers and Vanrolleghem (1995) proposed a method for the determination of endogenous decay parameter coupled with the growth rate from a single respirogram. Avcıoğlu *et al.* (1998) also argued that the OUR levels induced by $b_H X_H$ were too low and likely to bring sensitivity and accuracy problems. Their experiments yielded scattered data, sometimes with cyclic variations that were attributed to sequential release and utilization of endogenous residues, making numerical evaluation almost impossible. They have defined another respirometric method that consisted of measuring changes in the $\mu_H X_H$ response. The method reflected the variation of X_H with time by always sustaining a constant $\hat{\mu}_H$ level during the course of the experiment. They obtained a significantly lower b_H value of around $0.1\ d^{-1}$ at 20°C for domestic sewage. van Loosdrecht and Henze (1999), in a comprehensive review on this subject, stated that what was simply defined as endogenous decay, could be in reality a spectrum of different mechanisms such as maintenance energy requirements, decay of cells, endogenous respiration, grazing by higher organisms, effect of toxic substances or adverse conditions that result in cell lysis. In this respect, aerobic digestion tests, like the one proposed by Ekama *et al.* (1986), may be subject to severe interference by either external particulate slowly biodegradable substrate, X_S enmeshed with biomass or by internal storage products, X_{STO}, included as a model component in some new models (Gujer *et al.*, 2000).

Experimental assessment of X_H

Identification of the viable fraction in activated sludge, X_H, requires an OUR measurement that reflects the balance between the utilization of the electron acceptor and the process rates for growth and endogenous decay. Henze (1986) used this relationship to determine the viable biomass in wastewater with the assumption that the maximum specific substrate utilization rate at 20°C is 1 e^-.eq. $(g\ VSS\ d)^{-1}$, corresponding to 133 $mgCOD\ (g\ VSS\ h)^{-1}$. A value of 150 $mgCOD\ (g\ VSS\ h)^{-1}$ was adopted for the evaluation of experimental results. A range of 0.5–2.0 e^-.eq $(g\ VSS\ d)^{-1}$ is reported in the literature as a function of substrate and temperature for different types of heterotrophic bacteria (McCarty, 1972). Ekama *et al.* (1986) suggested that active biomass could be expressed in terms of the initial OUR value in the experiment for endogenous decay rate, provided that the values of b_H and f_{EX} are known. Similarly, as part of the high F/M ratio batch test for the assessment of $\hat{\mu}_H$, Kappeler and Gujer (1992) directly calculated X_H as a function of the initial OUR provided that $\hat{\mu}_H$, b_H, Y_H and f_{EX} are previously estimated. Ubisi *et al.* (1997) proved that the active heterotrophic biomass estimated with batch tests is in good agreement with the one calculated theoretically using steady state equations (Scheer *et al.*, 1996). Recently, Sözen *et al.*

(1998) used two batch OUR tests conducted at different F/M ratios and evaluated the viable fraction of the biomass by calibrating the initial OUR plateau of the test suggested by Ekama *et al*. (1986) with the $\hat{\mu}_H$ value computed from a similar test conducted at a much higher F/M ratio as defined by Kappeler and Gujer (1992).

Experimental assessment of k_h and K_X

Hydrolysis of the slowly biodegradable substrate, X_S, is a very important step of activated sludge modeling, because X_S accounts, as previously mentioned, for the bulk of the biodegradable COD content of both domestic sewage and industrial wastewaters. Depending upon their nature and size, different organic compounds lumped into this fraction undergo a sequence of complex reactions. As identification and description of these reactions in wastewater is impossible, they are conveniently collected into an overall single hydrolysis mechanism defined by means of a surface-limited reactions kinetics, with k_h and K_X as the rate parameters. Experimental assessment of the parameters concerning the surface saturation type hydrolysis was first proposed by Ekama *et al*. (1986) using the "square feed" method that depends upon OUR measurement. In addition, batch respirometric tests are proposed by adjusting the appropriate wastewater/biomass mixtures (Orhon *et al*., 1999c). For this purpose, the model equations are made operational with known values of COD fractions and kinetic parameters, either defined by direct experiments or adopted by experience prior to curve fitting with the selected OUR profile. Many researchers argued that it might be difficult and sometimes misleading to characterize the entire slowly biodegradable COD fraction by a single hydrolysis rate and suggested a dual hydrolysis mechanism for rapidly hydrolysable COD, S_H, and slowly hydrolysable COD, X_S, as different model components (Orhon *et al*., 1999c; Sollfrank and Gujer, 1991; Spanjers and Vanrolleghem, 1995).

Interpretation of the OUR profiles

For the interpretation of the OUR data, acetate, a simple well studied readily biodegradable organic compound, and domestic sewage were selected as typical substrates. A model simulation approach, using default values listed in Table 3, was adopted for acetate and domestic sewage. Random noise of 0.5 $mgO_2l^{-1}\,h^{-1}$ was added to approximate an experimental OUR profiles with selected characteristics. Model evaluation of the OUR data was performed using AQUASIM computer program (Reichert *et al*., 1998).

Table 3 Wastewater characteristics adopted for the model simulations

Parameter	Unit	Acetate	Domestic sewage	Textile effluent
Maximum heterotrophic growth rate, $\hat{\mu}_H$	Day^{-1}	7.0	6.0	3.2
Half saturation parameter for growth, K_S	mg COD l^{-1}	4	20	20
Heterotrophic yield parameter, Y_H	mg cell COD (mg COD)$^{-1}$	0.78	0.67	0.67
Endogenous decay parameter, b_H	Day^{-1}	0.2	0.2	0.14
Inert fraction of endogenous biomass, f_E	–	0.2	0.2	0.2
Maximum hydrolysis rate parameters, k_h	Day^{-1}	–	3.0	1.0
Half saturation parameters for hydrolysis, K_X	mgCOD (mg cell COD)$^{-1}$	–	0.1	0.16
Initial readily biodegradable COD, S_{S0}	mg COD l^{-1}	344	54	60
Initial slowly biodegradable COD, X_{S0}	mg COD l^{-1}	–	216	280
Initial active heterotrophic biomass, X_{H0}	mg cell COD l^{-1}	430	200	200
F/M Ratio	mg COD (mg cell COD)$^{-1}$	0.80	1.35	1.70

Relationship between endogenous decay and microbial growth

The measured OUR profile reflects the true electron acceptor utilization response of the microbial community under the operating conditions of the experimental reactor. This response is interpreted in terms of a model that assumedly describes the kinetic behavior of the community. For this purpose, kinetic parameters related to growth and endogenous decay, namely $\hat{\mu}_H$ and b_H, are generally determined beforehand, by means of differently structured OUR experiments, and incorporated into the model to fit the experimental OUR data. $\hat{\mu}_H$ and b_H are correlated by the active biomass concentration in the reactor, X_H which is difficult to determine at the start of the experiment. As the initial OUR value before substrate addition reflects $b_H X_H$, and after the substrate addition, $\mu_H X_H$, the OUR curve is fitted by searching for the most appropriate X_H value that correlates the two kinetic parameters, b_H and$\hat{\mu}_H$. As previously mentioned, it is also difficult to come up with an accurate assessment of b_H and $\hat{\mu}_H$ and the values experimentally computed may not correctly reflect the response of the microbial community to the selected wastewater. Often, values of these parameters are adopted from another source that is not compatible with the wastewater under investigation. The degree of uncertainty involved may seriously affect the interpretation of the OUR data, both for the relationship between endogenous decay and microbial growth and for the following hydrolysis phase.

An important observation in this respect is the fact that the OUR profile is sensitive to the chosen values of b_H and $\hat{\mu}_H$ and consequently, any change from the intrinsic values of the kinetic and stoichiometric parameters is likely to affect the profile. With all other parameters kept constant, a b_H value different than the one selected to characterize the

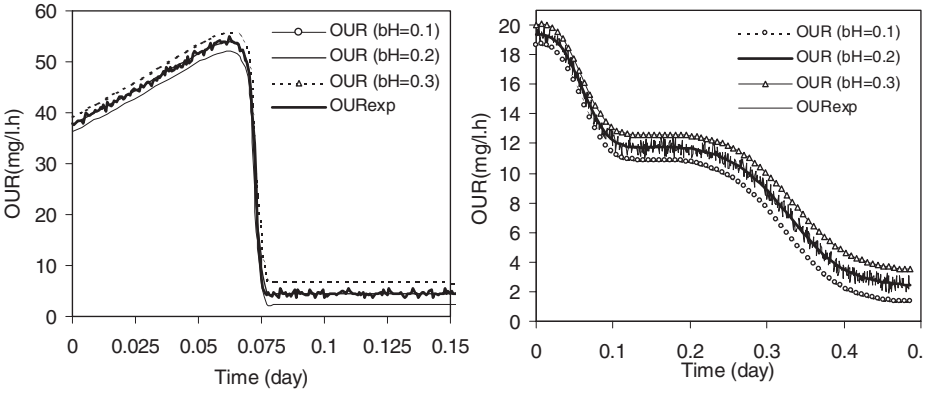

Figure 3 Effect of b_H on the OUR profile of acetate (left) and domestic sewage (right)

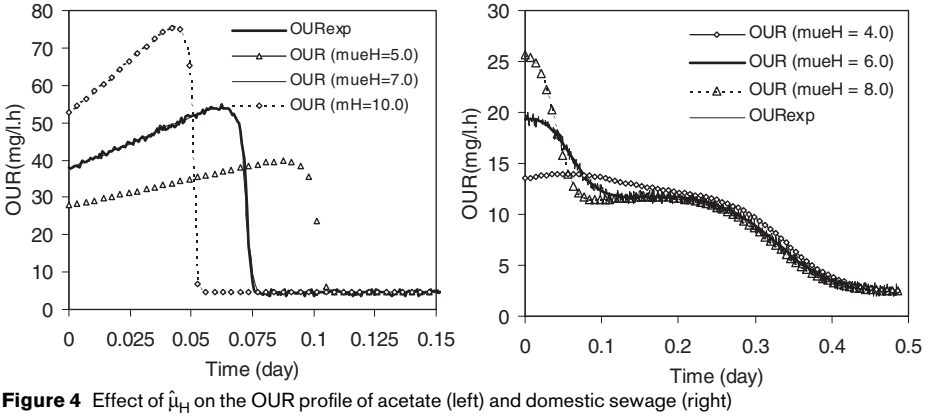

Figure 4 Effect of $\hat{\mu}_H$ on the OUR profile of acetate (left) and domestic sewage (right)

wastewater shifts the total OUR curve as shown in Figure 3. Similarly, a different $\hat{\mu}_H$ value significantly changes the shape of the plateau, while the area under the curve is conserved as illustrated in Figure 4. These discrepancies from the experimental data may easily be detected if both b_H and $\hat{\mu}_H$ are previously known. The main problem remains in the fact that with one of the parameters determined or adopted with a certain degree of error, there is the possibility of fitting the OUR profile by adjusting other parameters to values that do not totally relate to the wastewater tested. Basically, the $b_H X_H - \hat{\mu}_H X_H$ correlation automatically selects an X_H level different from the true value, and this will directly reflect on the values of other kinetic parameters. The effect of b_H and $\hat{\mu}_H$ on the estimation of other parameters is illustrated with model simulations outlined in Tables 4 and 5. In the case of acetate, this effect reflects a straightforward relationship between b_H and $\hat{\mu}_H$. For more complex wastewaters such as domestic sewage, it also involves hydrolysis parameters. Inspection of Table 4 shows that adoption of a b_H value between 0.1 – 0.3 d^{-1} leads to a significant $\hat{\mu}_H$ variation in the range of 2.56 – 10.47 d^{-1} for domestic sewage. The corresponding K_S variation remains negligible for domestic wastewater.

It should be remembered that the parameter estimation procedure minimizes the objective function of χ^2. As shown in Table 4 for domestic wastewater, estimation of the default values of the kinetic parameters corresponds to χ^2 of 47.40 and 68.54, respectively. OUR profiles generated by model simulation for the b_H range of 0.1 – 0.25 d^{-1} yield reasonably close χ^2 values and consequently fit well with the default OUR curve (Figure 5), despite the fact that they provide totally misleading information about the magnitude of the growth kinetics.

Table 4 The effect of b_H on the estimation of other kinetic parameters

	Parameter	Initial active biomass, X_{H0}	Growth kinetics		Hydrolysis kinetics		χ^2
	b_H value (d^{-1})	(mgCOD.l^{-1})	$\hat{\mu}_H$ (d^{-1})	K_S (mg.l^{-1})	k_h (d^{-1})	K_X –	
Acetic	0.30	311	9.12	5.87	–	–	501.13
Acid	0.25	359	8.11	4.89	–	–	174.42
	0.20*	427	7.02	4.12	–	–	47.40
	0.15	537	5.79	3.12	–	–	187.82
	0.10	779	4.18	2.23	–	–	715.36
Domestic	0.30	111	10.47	20.51	4.71	0.19	129.84
Wastewater	0.25	146	8.07	19.92	3.83	0.14	83.36°
	0.22	176	6.80	20.43	3.32	0.12	70.72
	0.20*	202	5.93	19.80	2.99	0.10	68.54
	0.17	254	4.78	20.03	2.46	0.08	74.24
	0.15	300	4.11	21.00	2.14	0.06	83.42
	0.13	363	3.44	21.31	1.82	0.05	96.28
	0.10	506	2.56	23.42	1.36	0.04	121.21°

* default used in the generation of the "true" data
° fit shown in Figure 5

Table 5 The effect of $\hat{\mu}_H$ on the estimation of kinetic parameters for $b_H = 0.2$ d^{-1} (domestic wastewater)

	Parameter	Initial active biomass, X_{H0}	Growth kinetics	Hydrolysis kinetics		χ^2
			K_S	k_h	K_X	
μ_H value	3.00	252	10.80	2.40	0.07	206.33
	5.00	221	15.30	2.73	0.08	90.09
	5.93	202	19.80	2.99	0.10	68.54
	7.00	189	26.05	3.19	0.11	84.15
	8.00	181	32.43	3.34	0.12	113.54

Assessment of the hydrolysis rate

Two major concerns exist in the experimental assessment of the hydrolysis parameters. The first concern is the effect of inaccurately pre-selected parameters on the evaluation of the OUR data. They lead to a distorted image of the hydrolysis process as far as the kinetic parameters are concerned. As given in Table 4, selection of a b_H value in the 0.13 – 0.25 d^{-1} interval induces a significant, yet statistically acceptable (χ^2 = 83.36 – 96.28) shift in the corresponding k_h levels in the range of 1.82 – 3.83 d^{-1}. A similar variation of 0.05 – 0.14 is also inflicted upon K_X.

At this point, it may be interesting to note the contrast between the minor impact of b_H on the OUR curve (Figure 3) and its significant implications on resulting information related to the hydrolysis kinetics. This can be explained on the basis of sensitivity analysis, indicating a uniform sensitivity for b_H throughout the entire OUR curve. Therefore, b_H can only be compensated by changing X_H, $\hat{\mu}_H$ and k_h parameters, where appropriate. From a modeling point of view, the effect of one parameter on a state variable (OUR) can be compensated by other parameter(s) when the parameter(s) has similar sensitivities in shape. In this situation, the shape of the sensitivity function for b_H is completely different from those of the others. However, since the parameter b_H is active during the entire OUR experiment, the change in b_H can only be balanced with relevant combinations of other parameters. Simulation studies also show that $\hat{\mu}_H$ exerts a similar but relatively limited impact on the hydrolysis parameters due to the rate limiting effect of the hydrolysis process after the first plateau (Table 5). This is also obvious from the sensitivity profiles where the effect of $\hat{\mu}_H$ and K_S on the OUR profile is negligible after the initial growth phase.

The second concern is the inherent property of the OUR test to generate not one, but a series of parameter couples for hydrolysis with equal statistical validity, even if all other kinetic and stoichiometric parameters are assessed or selected properly. In statistical terms, a confidence domain for the parameter couple with a given χ^2 value may be defined for each experimental OUR data set. Figure 6 gives the confidence ellipses delineated for different χ^2 contours for an OUR test run with an F/M ratio of 1.35 gCOD(g cell COD)$^{-1}$ for domestic sewage. If χ^2 = 128 is visually estimated to be the limit for conformity with the experimental OUR profile, then all k_h–K_X couples within the χ^2 = 128 contour may secure model calibration (Figure 7). In a more explicit way, k_h =3.2 d^{-1};K_X =0.13 and k_h = 2.8 d^{-1}; K_X = 0.075 define the limits of the χ^2 = 128 contour.

It is usually helpful to run a second experiment at a different preferably lower F/M ratio. Figure 8 gives a model evaluation for F/M = 0.33 gCOD(gcellCOD)$^{-1}$ obtained by

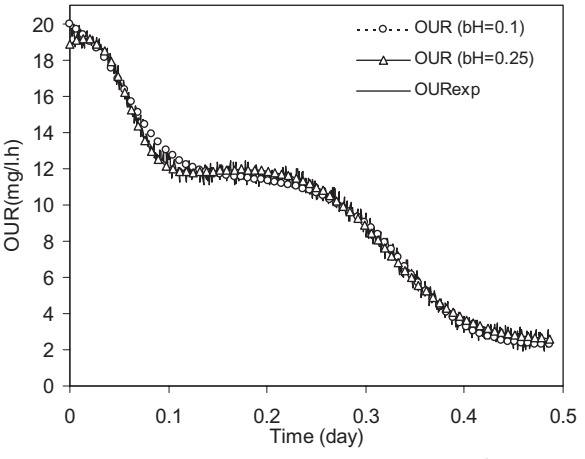

Figure 5 Fitting the OUR profile for domestic sewage for b_H 0.1 and 0.25 d^{-1}

increasing X_H to 800 mgl^{-1}. The figure also includes the results obtained with F/M = 1.35 gCOD(gcellCOD)$^{-1}$ for comparison. It is important to note that a lower F/M ratio is much more informative in the sense that it gives smaller areas within the same χ^2 contours; also the confidence regions are more inclined towards the k_h axis and therefore indicate observed more sensitive for K_X. The χ^2 contours rotate, as may be depicted in Figure 8 around the *optimum point* for the model simulation in this study.

This rotation property may be used to identify the optimum k_h–K_X couple that is associated with the wastewater tested, by means of two sets of OUR data generated at different F/M ratios (Sperandio and Paul, 2000; Orhon *et al*., 2001; Insel *et al*., 2002). However, this approach does not always yield the same optimum parameter estimates for two experiments with different initial conditions. A multi-experimental fitting procedure (Dochain and Vanrolleghem, 2001) has to be performed in order to acquire joint solution.

Evaluation and conclusion

The above evaluations of the model in relation to the available experimental data fit into the major question "Why do we use models?" for activated sludge systems. If the answer is performance calibration and prediction, then the next equally important issue is to ascertain which parameter, whether it be a model component or a kinetic parameter, has priority for

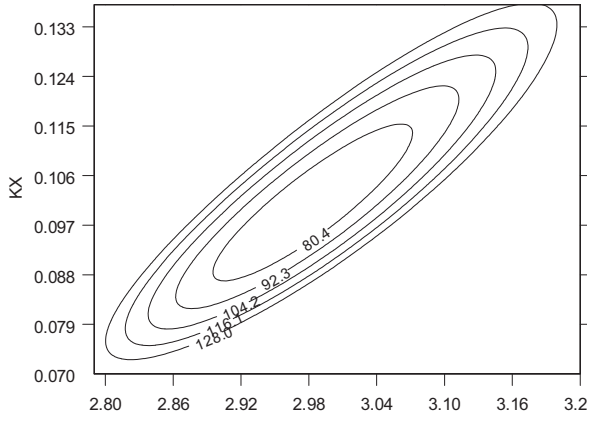

Figure 6 χ^2 contour plots for the estimation of k_h and K_X for F/M = 1.35 g COD(gcellCOD)$^{-1}$

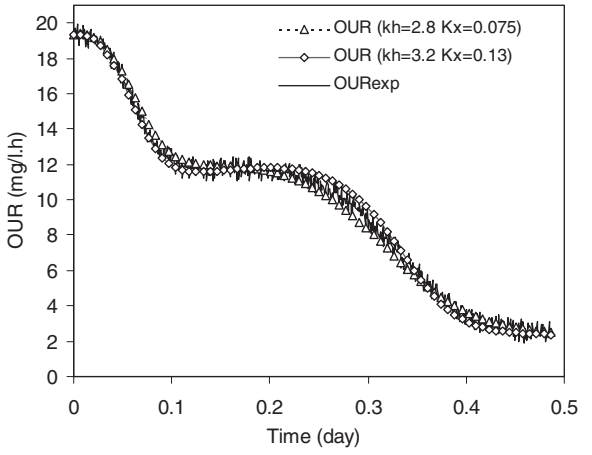

Figure 7 Simulation results for limiting k_h–K_X values for $\chi^2 = 128$ ($b_H = 0.2$ d^{-1})

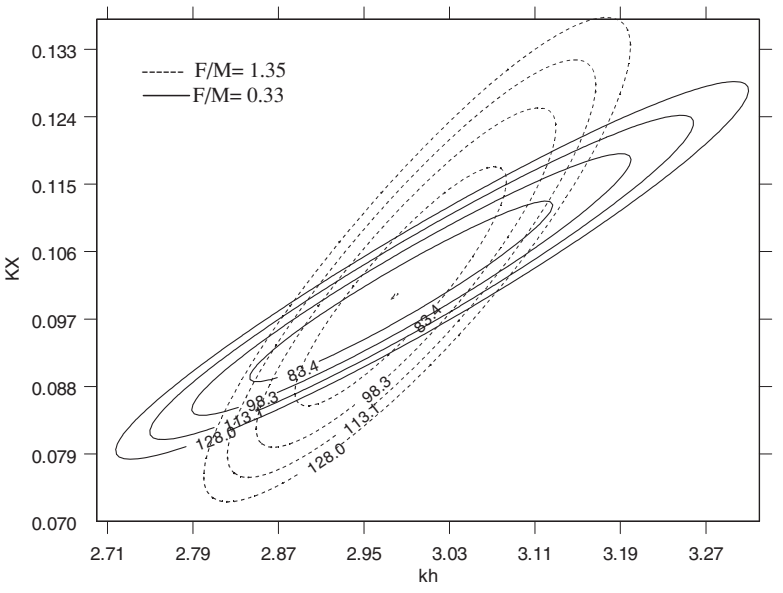

Figure 8 χ^2 contour plots for k_h–K_X values obtained with F/M ratios of 0.33 and 1.35 g COD(gcellCOD)$^{-1}$

that purpose and to give emphasis on its accurate assessment for the specific wastewater of concern. A modeling exercise, without the experimental assessment complement as exemplified in this study, may show that a given parameter may be assigned a secondary significance and adopted without too much concern as to whether it reflects the intrinsic process property. The paper addressed this problem as one of the major pitfalls in modeling, in the sense that an unjustifiable flexibility or a bias in one parameter may cause a significant propagation towards all parameters to be estimated from the experiment. Depending upon the modeling purpose, this problem should be given prime consideration as it exerts a direct influence on the prediction of overall performance of an activated sludge system.

For most effluents from agro-industries, hydrolysis of slowly biodegradable substrate appears to be the major issue. Respirometry, while very useful and promising, still involves a number of pitfalls for an accurate assessment of the rate parameters for this process. Moreover, all other parameters play a major role in the evaluation process and a joint estimation should be preferred over a sequential estimation.

Despite the important body of knowledge and experience now available, experimental evaluation still requires substantial additional effort for improvement. Sensitivity analysis and optimal experimental design techniques should be given major emphasis in this endeavor.

References

Avcıoğlu, E., Orhon, D. and Sözen, S. (1998). A new method for the assessment of heterotrophic endogenous respiration rate under aerobic and anoxic conditions. *Wat. Sci. Tech.*, **38**(8–9), 95–103.

Dochain, D., Vanrolleghem, P. and Van Daele, M. (1995). Structural identifiability of biokinetic models of activated sludge respiration. *Water Res.*, **29**, 2571–2579.

Dochain, D. and Vanrolleghem, P.A. (2001). *Dynamical Modelling and Estimation in Wastewater Treatment Processes*, IWA Publishing, London.

Dold, P.L., Ekama, G.A. and Marais, G.v.R. (1980). A general model for the activated sludge process. *Prog. Wat. Tech.*, **12**(6), 47–54.

Ekama, G.A. and Marais, G.v.R. (1979). Dynamic behaviour of the activated-sludge Process. *J. Water. Pollut. Con. F.*, **51**(3), 534–556.

Ekama, G.A., Dold, P.L. and Marais, G.v.R. (1986). Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Wat. Sci. Tech.*, **18**(6), 91–114.

Germirli, F., Orhon, D. and Artan, N. (1991). Assessment of the initial inert soluble COD in industrial wastewaters. *Wat. Sci. Tech.*, **23**(4–6), 1077–1086.

Grady, C.P.L. Jr., Smets, B.F. and Barbeau, D.S. (1996). Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. *Wat. Res.*, **30**, 742–748.

Gujer, W., Henze, M., Mino, T. and van Loosdrecht, M. (2000). Activated Sludge Model No.3. In: *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*, Henze, M., Gujer, W., Mino, T., van Loosdrecht, M. (eds.) IWA Scientific and Technical Report No.9. IWA London.

Henze, M., Grady, C.P.L. Jr., Gujer, W., Marais, G.v.R. and Matsuo, T. (1987). Activated Sludge Model No. 1, IAWPRC Scientific and Technical Report No. 1, IAWPRC, London.

Henze, M. (1992). Characterization of wastewater for modeling of activated sludge process. *Wat. Sci. Tech.*, **25**(6), 1–15.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C. and Marais, G.v.R. (1995). *Activated Sludge Model No.2. IAWQ Scientific and Technical Report, No.3*, IAWQ, London. ISBN 1 900222 00 0.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C., Marais, G.v.R. and van Loosdrecht, M.C.M. (1999). Activated Sludge Model No.2d, ASM2D, *Wat. Sci. Tech.*, **39**(1), 165–182.

Henze, M. (1986). Nitrate versus oxygen utilization rates in waste-water and activated-sludge systems. *Wat. Sci. Tech.*, **18**(6), 115–122.

Insel, G., Vanrolleghem, P.A and Orhon, D. (2002). Application of optimal experimental design for the assessment of hydrolysis kinetics under aerobic conditions (in preparation).

Kappeler, J. and Gujer, W. (1992). Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modeling. *Wat. Sci. Tech.*, **25**(6), 125–139.

Keesman, K.J., Spanjers, H. and van Straten, G. (1998). Analysis of endogenous process behaviour in activated sludge, *Biotech. Bioeng.*, **57**(2), 155–163.

McCarty, P.L. (1972). Stoichiometry of biological reactions. *Prog. Wat. Technol.*, **7**(1), 157–173.

Novak, L., Larrea, L. and Wanner, J. (1994). Estimation of maximum specific growth rate of heterotrophic and autotrophic biomass: A combined technique of mathematical modeling and batch cultivations. *Wat. Sci. Tech.*, **30**(11), 171–180.

Orhon, D., Artan, N., Buyukmurat, S. and Gorgun, E. (1992). The effect of residual COD on the biological treatability of textile wastewaters. *Wat. Sci. Tech.*, **26**(3–4), 815–825.

Orhon, D. and Artan, N. (1994). *Modeling of Activated Sludge Systems*, Technomic Publ. Co., Lancaster, USA.

Orhon, D., Taşlı, R. and Sözen, S. (1999a). Experimental basis of activated sludge treatment for industrial wastewaters – the state of the art. *Wat. Sci. Tech.*, **40**(1), 1–11.

Orhon, D., Karahan, Ö. and Sözen, S. (1999b). The effect of residual microbial products on the assessment of the particulate inert COD in wastewaters. *Wat. Res.*, **33**(14), 3191–3203.

Orhon, D., Ubay Çokgör, E. and Sözen, S. (1999c). Experimental basis for the hydrolysis of slowly biodegradable substrate in different wastewaters. *Wat. Sci. Tech.*, **39**(1), 87–95.

Orhon, D., Okutman, D. and Insel, G. (2001). Characterization and biodegradation of settleable organic matter for domestic wastewater, *WaterSA* (submitted).

Pearson, E.A. (1968). Kinetics of biological treatment in *Advances in Water Quality Improvement, Vol. 2*, E.F. Gloyna and W.W. Eckenfelder, Jr., eds., Austin, TX: University of Texas Press.

Petersen, B., Gernaey, K., Henze, M. and Vanrolleghem, P.A. (2001). Calibration of activated sludge models: A critical review of experimental designs. In: *Biotechnology for the Environment*. Eds. Agathos, S. and Reineke, W., Focus on Biotechnology, Vol. 3. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 80. (in Press).

Reichert, P., Ruchti, J. and Simon, W. (1998). Aquasim 2.0 Swiss Federal Institute for Environmental Science and Technology (EAWAG), CH-8600 Duebendorf, Switzerland.

Scheer, H. and Seyfried, C.F. (1996). Enhanced biological phosphate removal: Modelling and design in theory and practice, *Wat. Sci. Tech.,* **38**(8–9), 95–103.

Sollfrank, U. and Gujer, W. (1991). Characterization of domestic wastewater for mathematical modelling of the activated sludge process. *Water Sci. Technol.*, **23**(4–6), 1057–1066.

Spanjers, H. and Vanrolleghem, P. (1995) Respirometry as a tool for rapid characterization of wastewater and activated sludge. *Wat. Sci. Tech.*, **31**(2), 105–114.

Spanjers, H., Vanrolleghem, P.A., Olsson, G. and Dold, P.L. (1998). *Respirometry in Control of the Activated Sludge Process: Principles*. IAWQ Scientific and Technical Report No. 7. London, UK.

Sperandio, M. and Paul, E. (2000). Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various So/Xo Ratios, *Wat. Res.*, **34**(4) , 1233–1246.

Sözen, S., Ubay Çokgör, E., Orhon, D. and Henze, M. (1998). Respirometric analysis of activated sludge behaviour – II. Heterotrophic growth under aerobic and anoxic conditions, *Wat. Res.*, **32**(2), 467–488.

Ubisi, M.F., Joodi, T.W., Wentzel, M.C. and Ekama, G.A. (1997). Activated sludge mixed liquor heterotrophic active biomass, *WaterSA*, **23**(3), 239–248.

van Loosdrecht, M.C.M. and Henze, M. (1999). Maintanence, endogenous respiration, lysis, decay and predation. *Wat. Sci. Tech.*, **39**(1), 107–117.

Vanrolleghem, P.A., Van Daele, M. and Dochain, D. (1995). Practical identifiability of a biokinetic model of activated sludge respiration. *Wat. Res.*, **29**, 2561–2570.

Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P. and Takacs, I. (1999). Estimating (combinations of) Activated Sludge Model No.1 parameters and components by respirometry. *Wat. Sci. Tech.*, **39**(1), 195–214.

Van Haandel, A.P.C, Ekama, G.A. and Marais, G.v.R. (1981). The activated sludge process 3 – single sludge denitrification. *Wat. Res.*, **15**, 1135–1152.

Weijers, S.R. and Vanrolleghem, P.A. (1997). A procedure for selecting the most important parameters in calibrating the activated sludge model no.1 with full-scale plant data. *Wat. Sci. Tech.*, **36**(5), 69–79.