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Stoichiometry and kinetics of hospital wastewater treatment in a submerged membrane bioreactor

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ABSTRACT

The present article deals with the calibration and validation of a biological model of SMBR for hospital wastewater treatment using respirometry. In a first part, the stoichiometric and kinetic parameters are estimated and validated using the experimental oxygen uptake rate (OUR) profiles from the sodium acetate degradation process, according two kinetic theories: one considering that microorganisms use the carbon reserve and easily biodegradable substrates simultaneously for growth; and the other that microorganisms use the carbon reserve only when easily biodegradable substrate is depleted. In this study, the first theory proved to be the most adequate to predict the experimental OUR profile. In the second part, the hospital wastewater degradation process simulated using the theory determined as the more suitable. The stoichiometric parameters obtained for acetate were used for the hospital wastewater COD fractionation process, considering simultaneous growth and substrate storage. These COD fractions and the stoichiometric parameters obtained for acetate were employed for the simulation process of hospital wastewater degradation, where only kinetic parameters were calibrated. Good correspondence was obtained between experimental data and the model outputs. The values obtained for kinetic parameters were different from those obtained for sodium acetate, evidencing the influence of the substrate nature. Through the calibration of stoichiometric and kinetic parameters using the proposed procedure, the activated sludge models proved their capacity and usefulness for the simulation of a hospital wastewater degradation process

Keywords: Hospital wastewater; Modeling; Respirometry; Submerged membrane bioreactor; Substrate storage

1. Introduction

Among the technologies used to treat wastewater, the Submerged Membrane Bioreactor (SMBR) has excellent prospects because of the possibility it provides for water reuse. The SMBR can be defined as a system that combines biological degradation of wastewater effluents with membrane filtration [1]. For many years, these systems have shown their effectiveness in the treatment of municipal and industrial wastewater [2,3]. The efforts to model such wastewater treatment systems have always targeted both the biological processes (treatment quality target) and the various aspects of engineering (cost effective design and operation) [4]. Activated Sludge Models (ASMs) are the most widely accepted and used models for MBR biological process simulation [5]. However, numerous applications of ASMs to specific WWTPs have demonstrated that the parameters

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of these models are not universal since some adjustments of parameter values are necessary. In most applications, plant operation, influent and sludge characteristics require some of the model parameters to be adjusted [6–8]. At present, the kinetic parameters of these models are generally determined for systems that treat municipal wastewaters. Nevertheless, in recent years the use of MBRs to study the treatment of wastewater contaminated with pharmaceutically active compounds (PhACs) has increased considerably [9–15]. It is well known that the effectiveness of MBRs in further removing micropollutants is linked to the system's ability to operate under non-conventional operational conditions (high sludge retention time (SRT), high biomass concentration, etc.), as operations at high SRT can favour the development of slow-growing bacteria, the presence of more diverse microbial communities with broader physiological capabilities and the adaptation to degrade specific organic compounds [11]. The biological activity of PhACs or powerful cleaners present in hospital wastewater may have an incidence on non-targeted organisms and can thus affect or modify the microorganisms' metabolism, as well as the values of some biokinetic parameters. Understanding and evaluating organic substrate utilization has always been a major concern in activated sludge systems. First, this information was mainly used to establish an accurate stoichiometric relationship between the substrate removed, the biomass generated and the oxygen consumed. Later, reliable interpretation of this mechanism became important in nitrogen removal systems with the understanding of the denitrification efficiency dependence on the extent of available organic carbon in the anoxic zone [16].

In that context, modellers have used respirometry as an efficient technique for the estimation of stoichiometric and kinetic parameters [17–20]. Nevertheless, this technique sometimes fails due to the interference of some complex processes: during respiration tests, high biomass yield coefficients are frequently obtained. Even if only soluble, readily biodegradable substrates such as acetate are added, it appears from respiration tests that this substrate includes a slowly biodegradable fraction [5]. This behaviour is the result of the substrate storage process. In the conventional activated sludge processes, the feed regime is highly variable and biomass is subjected to alternating conditions of external substrate availability (feast phase) and absence (famine phase). Under these fluctuating conditions, internal storage polymers play an important role in the substrate consumption [21,22]. This process was included in the ASM3 model by Henze [5], although it has been widely criticized by many authors [23], mainly because it considers that all substrate is first stored, and that growth only occurs on stored substrate, when, in fact, there is evidence of storage and direct substrate consumption occurring simultaneously [23–28].

The present article deals with the calibration and validation of a biological model of SMBR for hospital wastewater treatment using respirometry. Considering simultaneous growth and substrate storage, two theories are evaluated, according to two kinetic theories: M1, which considers that microorganisms use the carbon reserve and easily biodegradable substrate simultaneously for growth, M2, which considers that microorganisms use the carbon reserve only when easily biodegradable substrate is depleted. A simplified version of the ASM3 model was used for this comparison. In this study the model obtained does not include any specific modification to account the pollutant effects. It only takes their influence on the stoichiometric and kinetic parameter values into account (through their induced presence).

To the best of our knowledge, this work is the first to simulate hospital wastewater treatment using an ASM model. For this purpose, the procedure of biodegradable COD fractionation, first proposed by Vanrolleghem et al. [34], is modified to consider the simultaneous growth and substrate storage.

2. Materials and methods

2.1. Experimental setup

Sludge samples from a SMBR installed in a hospital (CHU Purpan) located in Toulouse, France, were used for the respirometric tests. A detailed description of this installation has already been given by Quesada et al. [14]. The respirometer (Fig. 1) consisted of a biological reactor with a working volume of 1.5 L.

The aeration was provided through a perforated tube placed at the bottom of the reactors. The mixture homogeneity was achieved by means of a mechanical stirrer. The air flow was controlled to maintain a dissolved oxygen concentration between 4.0 and 5.0 mg L^{-1}. The temperature was controlled at 26°C and the oxygen concentration was measured continuously by oxygen electrodes (YSI 5739, with an actual temporal resolution of 1.5s) connected to an oximeter YSI MODEL 57. The oxygen concentration was recorded continuously with a computer. This device allowed the dissolved oxygen concentration of the biological suspension to be continuously monitored during the substrate degradation.

2.2. Respirometric tests

2.2.1. Using sodium acetate as carbon source

One part of the easily biodegradable substrate is used for the biomass synthesis, including storage, while the other part is used as an energy source. This part is oxidized through the respiration process for energy production, which is necessary for biomass synthesis, cell maintenance, transport, etc. The oxygen consumption associated with the degradation of a well-known quantity of easily biodegradable substrate was determined. For this determination, Allylthiourea (ATU, 15 mg L^{-1}) was added to avoid nitrification, and sodium acetate was used as the readily biodegradable organic substrate (CH_3COONa, 7000 mg L^{-1}). The COD concentration was modified in each experiment by adding different volumes of substrate. Table 1 shows the experimental conditions for the respirometric tests.

2.2.2. Using hospital wastewater as carbon source

The same procedure as described for acetate was carried out but, in this case, two samples of 200 mL of hospital wastewater were studied using respirometric tests. The first

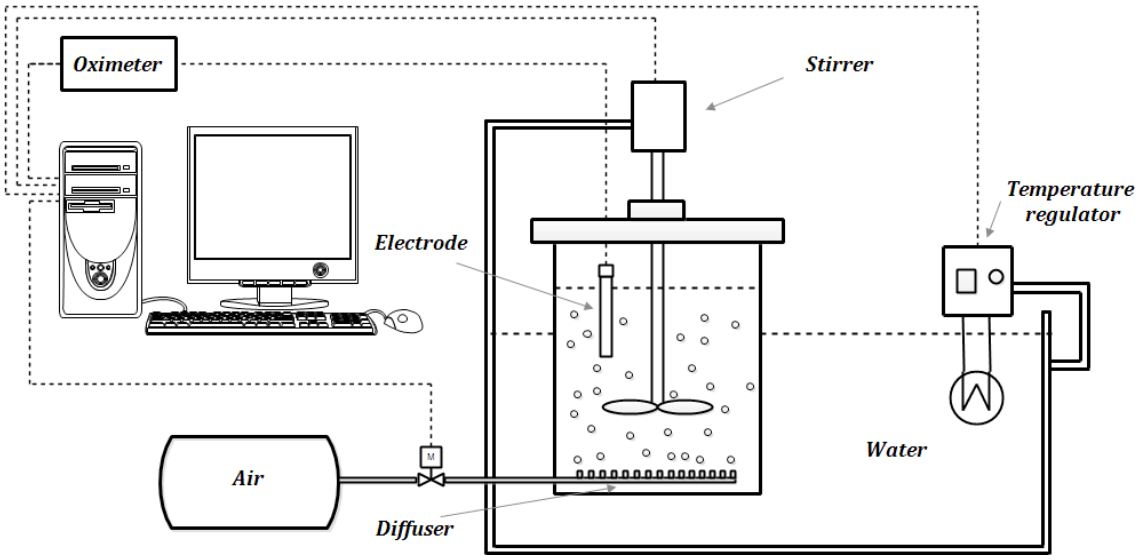

Fig. 1. Experimental setup for respirometric tests.

Table 1
Experimental conditions of respirometric tests carried out to determine the heterotrophic yield

No.	Sludge volume (L)	Acetate volume (mL)	CH_3COONa (mgCOD L^{-1})
1	1.56	30	103
2	1.50	50	176
3	1.50	70	243
4	1.50	90	309
5	1.50	130	435

sample was filtered using a 1.2 µm fiberglass filter, while the second one was untreated. The total COD was determined for each sample using the spectrometric methods with reagent kits (HACH LANGE kits of LCI 500 or LCK 514 for COD). The undegradable soluble matter was assumed to be source of 90% of permeate COD [33].

The soluble biodegradable and the slowly biodegradable substrates were estimated by combining modelling and experimental OUR data. The particulate undegradable matter was obtained by a mass balance (detailed in section 3.2). Again, allylthiourea was added for the determination of the heterotrophic biomass activity only. For modelling, the biomass amount was considered as negligible in the influent wastewater and alkalinity was discarded because pH in the bioreactor was kept neutral.

3. Modelling

3.1. Stoichiometry of easily biodegradable substrate degradation process

Since storage may occur in biomass of conventional activated sludge processes, it would be more likely to occur with a more fluctuating process: SMBR, treating unusual wastewater such as hospital wastewater. Several models that consider simultaneous growth and substrate storage have been proposed as alternatives to ASM3 [23–29]. Under dynamic conditions, growth of biomass and storage of polymers occur simultaneously when there is an excess of external substrate (feast period). Once all the external substrate is consumed, stored polymers can be used as a carbon and energy-source (famine period). The polymer acts as a reserve for the substrate that is taken up but not directly used for growth. In this way, growth of biomass can continue at a similar or slightly decreased rate in periods when there is no external substrate supply. The whole mechanism enables the bacteria to maintain their growth at a more or less constant, or balanced, rate and efficiently compete for substrate under dynamic substrate supply [34]. In this work, the substrate consumption and biomass growth mechanism occurring in an activated sludge system under aerobic condition was described using a modification of ASM3. The proposed model considers that under feast conditions, part of the substrate is used directly for biomass growth, while the rest is stored as internal storage products. The stored products are consumed to produce biomass with a kinetics different from that of direct use (Fig. 2). Taking the above analysis into account, the stoichiometry of easily biodegradable organic substrate was established (Fig. 3).

3.2. Influent wastewater fractionation considering simultaneous growth and substrate storage

Many methods of influent fractionation are used for wastewater degradation simulation, usually based on respirometric tests. These tests combine modelling with experimental data, and generally, the subprocesses are studied in isolated systems. For example, easily biodegradable substrate and slowly biodegradable substrate are determined by means of a respirometric test, in which only the heterotrophic biomass activity is evaluated using ATU as autotrophic biomass activity inhibitor [35].

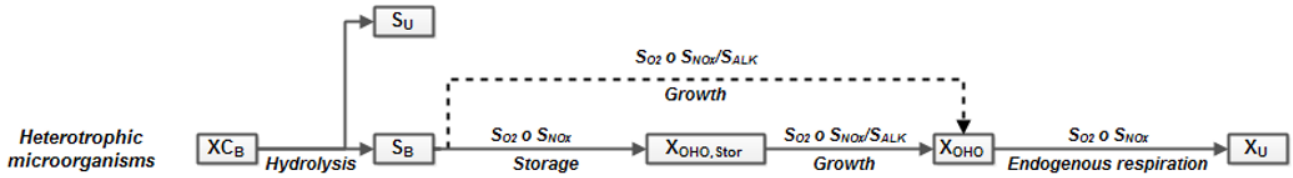

Fig. 2. Metabolic routes of biological degradation of carbonic matter for heterotrophic microorganisms.

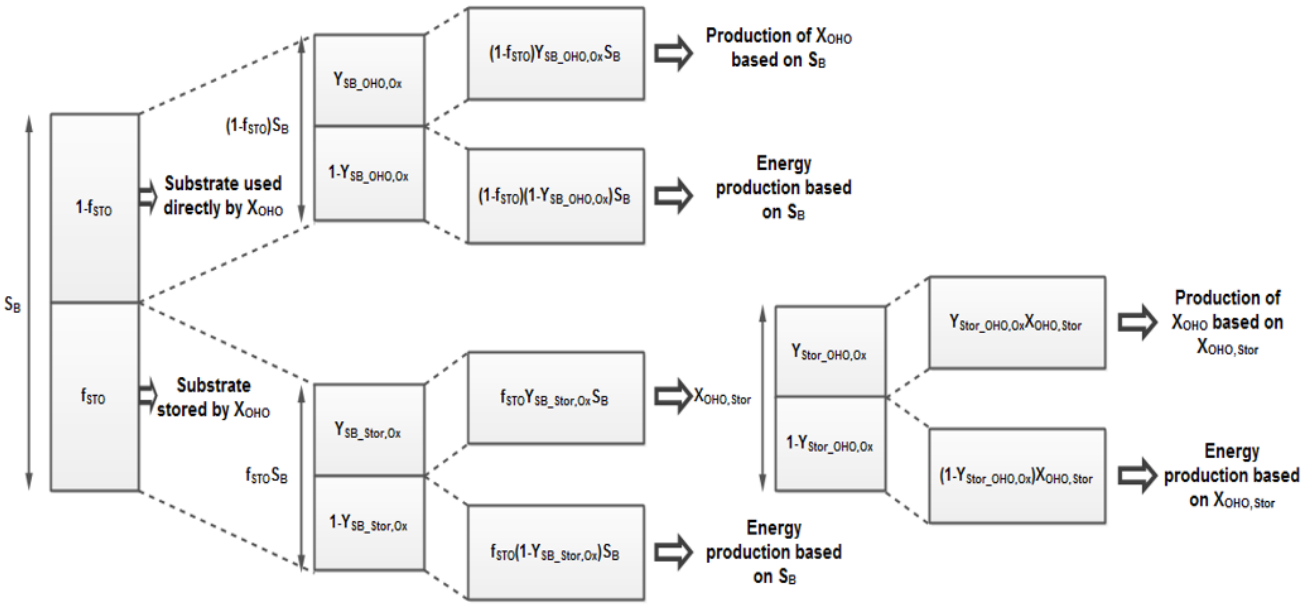

Fig. 3. Stoichiometry of the easily biodegradable organic substrate consumption.

In recent years, simultaneous growth and substrate storage has been studied and modeled by many authors using the ASM3 modification [16,24,27,29,30,36–39]. Nevertheless, a standard procedure for determining the S_B and XC_B fractions taking this phenomenon into account does not exist to date. In this study, the respirometric method for S_B and XC_B estimation proposed by Vanrolleghem et al. [32] was adapted. Its graphical method for separating the S_B and XC_B areas was employed and the expressions used for the calculation of these fractions were modified, taking the storage phenomenon into consideration. Fig. 4 shows the procedure followed for the determination of the oxygen consumption for S_B and XC_B degradation taking simultaneous growth and substrate storage into account.

As in Jiang et al. [40], a straight line was fitted to the last part of the tail (from 48 to 120 min) to differentiate between S_B and XC_B. Consequently, S_B was calculated from the area between the total OUR curve and the extended fitting line (0–48 min), while XC_B was calculated from the area between the extended fitting line (0–48 min) and the endogenous respiration line plus the area between the total OUR curve and the endogenous respiration line (48–120 min). Note that, in this case, the S_B and XC_B areas were determined using the graphical method proposed by Vanrolleghem et al. [32], but the expressions for the determination of the aforementioned fractions were modified, including the storage process.

In Fig.4, the area that corresponds to the oxygen consumed for easily biodegradable substrate degradation shows a small tail at the end of the degradation, unlike what is found for a system in which substrate storage does not take place. This tail is associated, not only with the low XC_B degradation rate as result of the XC_B hydrolysis, but also with other processes such as substrate storage and the degradation of stored material.

Thus, and taking the stoichiometry of the easily biodegradable substrate into consideration (Fig. 3), the following oxygen consumption balances for the individual processes can be performed:

Storage

$$OC_{Stor} = \left(1 - Y_{SB\_Stor,Ox}\right) f_{STO} S_B \quad (1)$$

Growth based on easily biodegradable substrate

$$OC_{G,SB} = \left(1 - Y_{SB\_OHO,Ox}\right)\left(1 - f_{STO}\right) S_B \quad (2)$$

Growth based on stored material

$$OC_{G,stor} = \left(1 - Y_{Stor\_OHO,Ox}\right) Y_{SB\_Stor,Ox} f_{STO} S_B \quad (3)$$

In this case, using the oxygen consumption balances of the individual processes involved in each substrate fraction

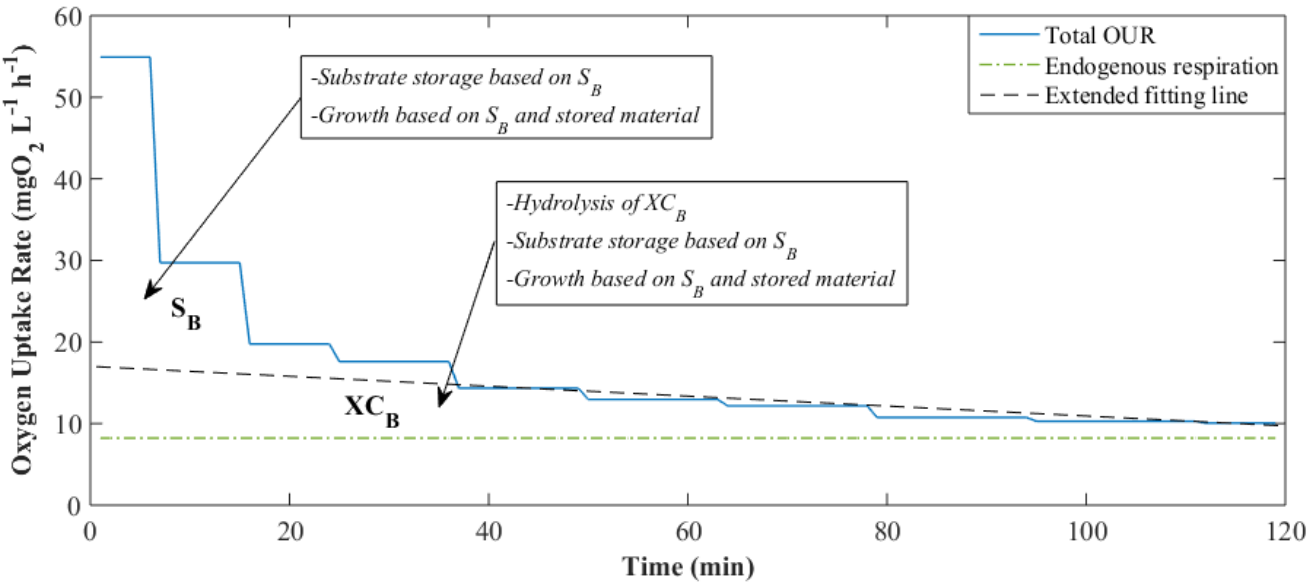

Fig. 4. Determination of the oxygen consumption for S_B and XC_B degradation taking into account simultaneous growth and substrate storage.

degradation, new expressions for estimating the S_B and XC_B fractions were developed:

$$XC_B = \frac{V_w + V_{sludge}}{V_w\left[1-\left(Y_{SB\_OHO,Ox} - f_{STO}Y_{SB\_OHO,Ox} + f_{STO}Y_{SB\_Stor,Ox}Y_{Stor\_OHO,Ox}\right)\right]}\Delta O_{2\_XCB} \quad (4)$$

$$S_B = \frac{V_w + V_{sludge}}{V_w\left[1-\left(Y_{SB\_OHO,Ox} - f_{STO}Y_{SB\_OHO,Ox} + f_{STO}Y_{SB\_Stor,Ox}Y_{Stor\_OHO,Ox}\right)\right]}\Delta O_{2\_SB} \quad (5)$$

Considering the total oxygen consumption and Eqns. (1), (2) and (3) the net heterotrophic yield can be expressed as:

$$Y_{SB\_OHO,Ox,net} = Y_{SB\_OHO,Ox} - f_{STO}Y_{SB\_OHO,Ox} + f_{STO}Y_{SB\_Stor,Ox}Y_{Stor\_OHO,Ox} \quad (6)$$

Note that knowledge of f_{STO}, $Y_{SB\_OHO,Ox}$, $Y_{SB\_Stor,Ox}$ and $Y_{Stor\_OHO,Ox}$ is needed for the calculation of S_B and XC_B from respiration rates. These parameters can be obtained by combining experimental OUR data and modelling. The particulate undegradable substrate was determined by means of COD balance in which the biomass concentration was neglected:

$$X_U = COD_T - \left(S_B + XC_B + S_U\right) \quad (7)$$

3.3. Kinetics of acetate degradation process

The model was implemented using the Petersen matrix notation (Table 2). In this case, only the heterotrophic microorganism degradation processes were taken into account. Two theories of kinetic degradation were compared (Table 2):

M1: The theory used for most authors that considers that microorganisms use the carbon reserve only when easily biodegradable is depleted [16,24,27,30,36–38].

M2: The theory proposed by Fan et al. [29] that considers that microorganisms use the carbon reserve and easily biodegradable substrates simultaneously for growth.

In this case, a simplified version of the ASM3 model, considering simultaneous growth and substrate storage, was used for the simulation of the hospital wastewater degradation process. The model obtained does not include any modification to consider the specific degradation of pollutants contained in the hospital wastewater, but their influence on the COD degradation is taken into account through the stoichiometric and kinetic parameter values obtained with the hospital wastewater.

3.4. Simulation strategy

Firstly, the sodium acetate degradation process was simulated using the M1 and M2 theories (Models of Table 2). For this purpose, the calibration and validation processes of both models were conducted using the experimental oxygen uptake rate profiles corresponding to the sodium acetate degradation samples 1 and 2, respectively, of Table 1. Then, the stoichiometric coefficients obtained for sodium acetate were used to determine the hospital wastewater COD fractions, using Eqns. (4), (5) and (7). These COD fractions and the stoichiometric parameters obtained for acetate were employed for the calibration of the kinetic parameters of hospital wastewater degradation, (minimum MRE, definition in the next section). Then all the values determined were used to simulate the degradation of the hospital wastewater.

3.5. Calibration process

For the calibration process, the two theories were implemented in Matlab 8.5, and an optimization process was carried out. To do this, the most sensitive parameters of

Table 2
Petersen matrix for the simplified version of the ASM3 model and the theories for the kinetic degradation of carbon substrate considering simultaneous growth and substrate storage for heterotrophic microorganisms in aerobic conditions

No	Process	S_{O2}	XC_B	S_B	S_U	S_{NHx}	X_U	X_{OHO}	$X_{OHO,Stor}$	Rate (ρ_i)	Theories	
											M1	M2
1	Hydrolysis		–1	$1–f_{SU\_XCB,hyd}$	$f_{SU\_XCB,hyd}$	$i_{N\_XCB}–(1–f_{SU\_XCB\_hyd})$ $i_{N\_SB}–f_{SU\_XCB\_hyd}i_{N\_SU}$				$q_{XCB\_SB,hyd}M_{XCB}X_{OHO}$	x	x
2	*Aerobic storage of $X_{OHO,Stor}$*	$–(1–Y_{SB\_Stor,Ox})$		–1		i_{N_SB}			$Y_{SB\_Stor,Ox}$	$f_{STO}q_{SB\_Stor}M_{SO2}M_{SB}X_{OHO}$	x	x
3	*Aerobic growth of X_{OHO} based on S_B*	$–(1–Y_{SB\_OHO,Ox})$ $/Y_{SB\_OHO,Ox}$		$–1/Y_{SB\_OHO,Ox}$		$i_{N\_SB}/Y_{SB\_OHO\_Ox}–i_{N\_XBio}$		1		$(1–f_{STO})\mu_{OHO,Max}M_{SO2}M_{SB}M_{SNHx}X_{OHO}$	x	x
4	*Aerobic growth of X_{OHO} based on $X_{OHO,Stor}$*	$–(1–Y_{Stor\_OHO,Ox})$ $/Y_{Stor\_OHO,Ox}$				$–i_{N\_XBio}$		1	$–1/Y_{Stor\_OHO,Ox}$	$\mu_{OHO,Max}M_{SO2}M_{Stor}M_{SNHx}X_{OHO}$		x
										$\mu_{OHO,Max}M_{SO2}M_{Stor}M'_{SB}M_{SNHx}X_{OHO}$	x	
5	*Aerobic respiration of X_{OHO}*	$–(1–f_{XU\_Bio,lys})$				$–f_{XU\_Bio,lys}i_{N\_XU}+i_{N\_XBio}$	$f_{XU\_Bio,lys}$	–1		$m_{OHO,Ox}M_{SO2}X_{OHO}$	x	x
6	*Aerobic respiration of $X_{OHO,Stor}$*	–1							–1	$m_{Stor,Ox}M_{SO2}X_{OHO,Stor}$	x	x

Processes [1–6] were taken into account for the simulation of wastewater degradation. Processes [2–6] were taken into account for the simulation of sodium acetate degradation. Rate expressions from processes [2–4] were modified according to the theory studied.

$$M_{SO2}=\frac{S_{O2}}{K_{O2,OHO}+S_{O2}};M_{SB}=\frac{S_B}{K_{SB,OHO}+S_B};M_{XCB}=\frac{\left(XC_B/X_{OHO}\right)}{K_{XCB,hyd}+\left(XC_B/X_{OHO}\right)};$$

$$M_{Stor}=\frac{\left(X_{OHO,Stor}/X_{OHO}\right)}{K_{Stor,OHO}+\left(X_{OHO,Stor}/X_{OHO}\right)};M'_{SB}=\frac{K_{SB,OHO}}{K_{SB,OHO}+S_B};M'_{Stor}=\frac{K_{Stor,OHO}}{K_{Stor,OHO}+\left(X_{OHO,Stor}/X_{OHO}\right)}$$

the model were selected and their values were estimated, minimizing the mean of the sum of the relative deviations between the measured data and the model predictions (MRE):

$$MRE=\frac{1}{n}\sum_{i=1}^{n}\left|\frac{OUR_{\exp,i}-OUR_{model,i}}{OUR_{\exp,i}}\right| \quad (8)$$

This function was minimized by taking, the parameters shown in Table 4 as independent variables, and the OUR values as response variable. The parameter values that were maintained constant during the calibration process are shown in Table 3.

The optimization process was carried out in Matlab 8.5 using the genetic algorithm. The initial concentration of heterotrophic microorganisms was estimated according to the method based on the baseline endogenous OUR level prior to substrate addition proposed by Sin et al. [41]:

$$OURend_{(0)}=-m_{OHO,Stor}\left(1-f_{XU\_Bio,lys}\right)X_{OHO(0)} \quad (9)$$

Table 3
Parameter values maintained constant during the calibration process

Symbol	Value	Reference
$f_{XU\_Bio,lys}$, gCODgCOD^{-1}	0.2	[5]
i_{N_XBio}, gNgCOD^{-1}	0.07	[5]
K_{O2_OHO}, $g_{O2}m^{-3}$	0.2	[5]
$m_{OHO,Ox}$, d^{-1}	0.2	[5]
$m_{Stor,Ox}$, d^{-1}	0.2	[5]
i_{N_XU}, gNgCOD^{-1}	0.02	[5]

4. Results and discussion

4.1. Parameter estimation combining experiment and modelling using acetate as carbon source

The parameter variation ranges and the numeric results of the calibration process for the experimental system studied using the two kinetic degradation theories are shown in Table 4.

Table 4
Numerical results of calibration and validation processes using acetate as carbon source

No	Type	Symbol	Range	Theories	
				M1	M2
1	*Stoichiometric parameters*	$Y_{SB\_OHO,Ox}$, gCODgCOD^{-1}	0.60–0.68	0.62	0.65
2		$Y_{SB\_Stor,Ox}$, gCODgCOD^{-1}	0.80–0.86	0.80	0.85
3		$Y_{Stor\_OHO,Ox}$, gCODgCOD^{-1}	0.60–0.68	0.66	0.66
4		f_{STO}, gCODgCOD^{-1}	0.25–1.00	0.66	0.60
5		i_{N_XU}, gNgCOD^{-1}	0.01–0.02	0.01	0.02
6	*Kinetic parameters*	q_{SB_Stor}, gCODgCOD^{-1}	1–15	1.45	1.55
7		$\mu_{OHO,Max}$, d^{-1}	1–6	2.11	2.03
8		K_{SB_OHO}, gCODm^{-3}	0.20–20	0.65	0.53
9		K_{Stor_OHO}, gCODgCOD^{-1}	0.001–1	0.40	0.13
10		K_{NHx_OHO}, gNm^{-3}	0.01–0.05	0.05	0.04
MRE of estimation of OUR for calibration (103 mgCOD L^{-1}), %				7.14	4.54
MRE of estimation of OUR for validation (176 mgCOD L^{-1})††, %				9.29	6.13

†See Fig. 5; ††see Fig. 6

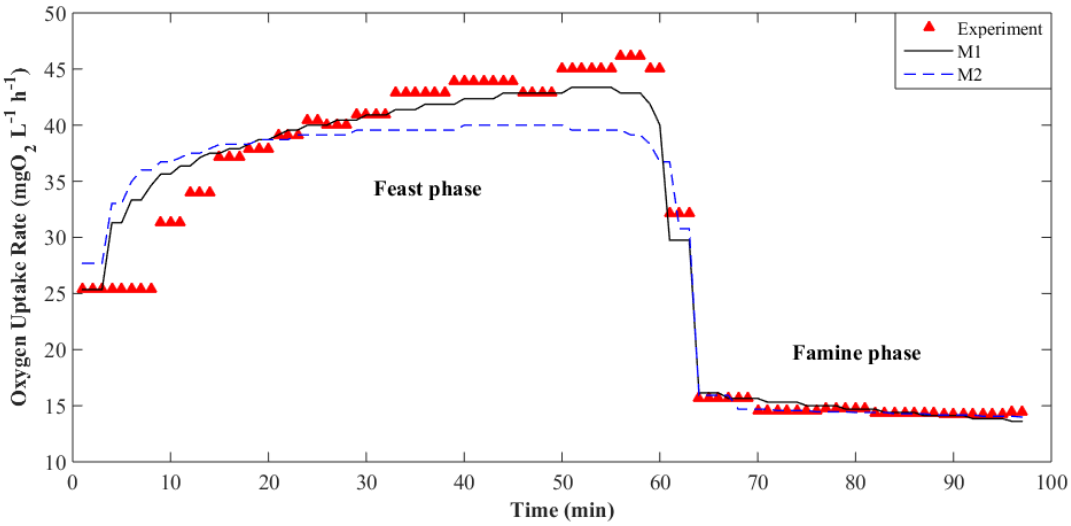

Fig. 5. Model calibration for two kinetic degradation theories considering simultaneous growth and substrate storage (103 mgCOD L^{-1} of sodium acetate) for M1 and M2 theories.

All the parameters are within their expected range. For the stoichiometric parameters which are the main concern of this part, even if only minor numerical differences are observed, it is important to consider their influence on the model, which means that slight variations of these parameters can lead to significant variations in the output variables of the calibrated model. Using the stoichiometric parameter values obtained for models M1 and M2 in Eq. (6), net heterotrophic yields of 0.56 and 0.60 gCOD gCOD^{-1}, respectively are obtained for the models. These values are lower than the standard values reported in the literature for systems in which the storage phenomenon does not take place (0.63–0.68 gCOD gCOD^{-1}), which is in agreement with the results obtained by Beun et al. [34]. At the same time, these results demonstrate the robustness of the mathematical calibration procedure, which gave results in accordance with the physical phenomena. Fig. 5 shows the calibration of OUR profiles for the two kinetic degradation theories, considering simultaneous growth and substrate storage.

The lowest mean relative estimation error was obtained for M2, with a value 4.54%. For M1, an underestimation of the OUR values was observed, which was more accentuated in the feast phase, and corresponded, in this case to simultaneous growth and substrate storage. During the famine phase, easily biodegradable substrate was not available and only stored material was consumed until endogenous respiration was reached.

Fig.6 shows the validation OUR profiles for the two kinetic degradation theories considering simultaneous growth and substrate storage, using the parameter values obtained during the calibration. In this case, a different COD value of sodium acetate was used during validation

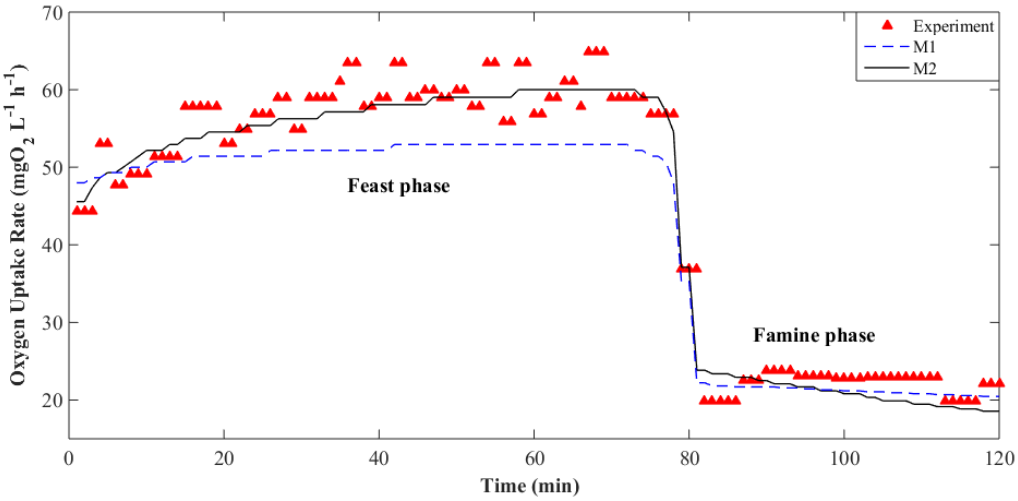

Fig. 6. Model validation for two kinetic degradation theories considering simultaneous growth and substrate storage (176 mgCOD L^{-1} of sodium acetate) for M1 and M2 theories.

process, in contrast to the approach of to Fan et al. [29] and Sin et al. [41] who carried out the validation process using a second pulse of sodium acetate with the same COD value. In fact, it is important to consider that, in real systems under dynamic conditions of feeding, the COD value changes regularly. As in the calibration process, during the validation, the M2 theory showed the best MRE of 6.13%. M1 showed an underestimation of the OUR profile during the feast phase while, during the famine phase, both models fitted the OUR profile adequately. The optimal calibrated parameters allowed the OUR profile to be simulate adequately with a different concentration of sodium acetate (176 mgCOD L^{-1}).

4.2. Simulation of separated processes for interpretation

Figs. 7 and 8 show the oxygen uptake rate and substrate behaviour for the individual processes during the acetate degradation using M2 theory for the calibration and validation experiments.

As can be seen in Figs. 7 and 8, the model predictions are in accordance with the assumptions used during the system modelling: simultaneous microorganism growth based on acetate and stored material during the feast period and microorganism growth based only on stored material during the famine period. In both experiments, it is noticeable that the OUR is mainly for growth based on acetate, then for endogenous respiration. In Figs.7 A and 8A, at the beginning of the aeration period, the OUR for the storage phenomenon is higher than the OUR for the growth based on stored material and, with the increase in stored substrate, the proportions are inversed at the end of the aeration period; this rise in OUR due to growth based on stored material explains the slight slope of the plateau of the total OUR, a phenomenon that is not considered in the M1 theory.

The proportion of OUR for growth based on acetate given by the value of stoichiometric parameters should be lower than that given by the simulation (around 1/3 of the OUR). This means that the kinetics of the reactions influence these results, the kinetics of the acetate degradation is fast and may not be suitable for other substrates. Low OUR values for the stored material degradation were also observed, which explains the OUR queue at the end of each experiment, when acetate was depleted and only stored material was available for microorganism growth and energy production.

4.3. Hospital wastewater characterization considering simultaneous growth and substrate storage

Using the numerical results obtained during the calibration process for M2 theory, the hospital wastewater was fractionated according to the methods explained in section 3.2. Specifically, the stoichiometric parameter values obtained for sodium acetate were used. Table 5 shows the results of the COD fractionation according to the ASM models using the adapted procedure.

According to the experimental results, the biodegradable substrate present in this wastewater is mainly composed of soluble and colloidal matter, where the amount of particulate slowly biodegradable substrate is smaller than that of easily biodegradable substrate. In this case, most of the biodegradable matter contained in the wastewater under study was in the form of particles with diameters smaller than 1.2 μm. Little difference was observed in the estimation of easily biodegradable substrate for filtered and non-filtered hospital wastewater (difference of 17 mgCOD L^{-1}, which represents 13% of the concentration of S_B obtained for the non-filtered hospital wastewater), when in fact the same concentration is expected for both samples. This slight difference may have been a result of the filtration process, where a small amount of easily biodegradable substrate might be absorbed by the filter. The high value obtained for undegradable soluble and particulate matter is in accordance with the kind of residual in the study, in which a considerable number of PhACs is present, as reported by Quesada et al. [14] for the same experimental setup.

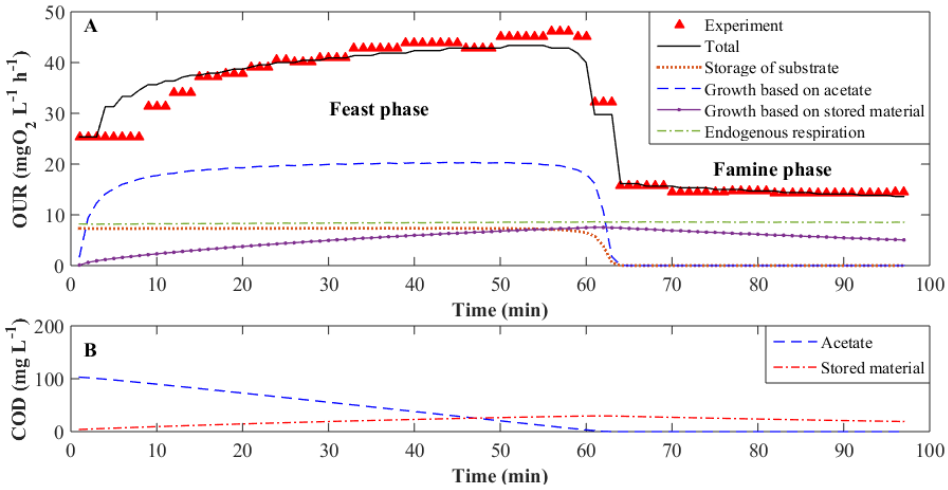

Fig. 7. Oxygen uptake rate (A) and substrate behaviour expressed as COD (B) for the individual processes during the acetate degradation for M1 theory (103 mgCOD L^{-1}).

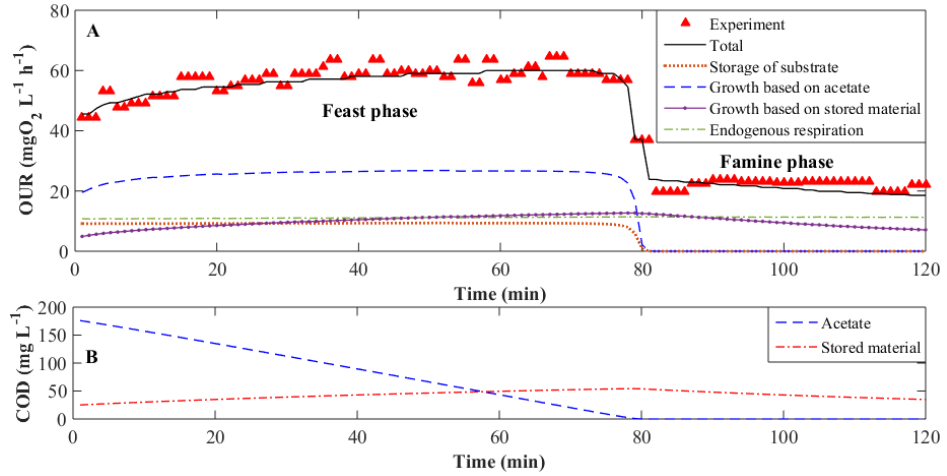

Fig. 8. Oxygen uptake rate (A) and substrate behaviour expressed as COD (B) for the individual processes during the acetate degradation for M1 theory (176 mgCOD L^{-1}).

Table 5
Fractionation of the COD of the hospital wastewater influent using the new procedure

Type of hospital wastewater	S_B (mgCOD L^{-1})	XC_B (mgCOD L^{-1})	S_U (mgCOD L^{-1})	X_U (mgCOD L^{-1})	Total COD (mg L^{-1})
Filtered	130	110	89	–	329
Non-filtered	147	126	89	202	564
Difference	17	16	0	202	235

4.4. Parameter estimation combining the experiments and modelling using hospital wastewater as carbon source

In this case, the calibrated parameters obtained for the best theory using acetate as the carbon source were evaluated in the model to simulate the degradation of 200 mL of filtered hospital wastewater (Table 5). The model did not achieve a sufficiently good fit with the experimental OUR data, so it was necessary to recalibrate the model to simulate the hospital wastewater degradation process. Since acetate is one of the pattern substrates used for the estimation of stoichiometric parameters in a procedure followed by several other authors [18,20,40,42,43], for the new calibration process, only the kinetic parameters were calibrated, and the stoichiometric parameter values obtained for acetate continued to be used. The validation process was car-

ried out using non-filtered hospital wastewater (Table 5). The values presented in Table 5 are the fraction concentrations in the influent hospital wastewater and, for the modelling process, these values were modified by dilution when added into the system volume. Table 6 shows the numerical results for the calibration process using filtered hospital wastewater as the carbon source.

The two parameters that increased significantly when changing from sodium acetate to hospital wastewater were q_{SB_Stor} and K_{SB_OHO}. Although they nearly compensated for one another in the case of storage of S_B, the influence was more marked on the slow-down of process 3 of Table 2, the growth of X_{OHO} based on S_B, which can be explained by a substrate with complex molecules (such as PhACs, cleaning products) being more difficult to transform into biomass. This result proves the influence of the substrate nature on the kinetic parameters of the system, and the great importance of calibrating these systems using the real feeding substrate. Çığgın et al. [16]. They obtained the same stoichiometry for all the conditions studied and found that only the kinetic parameters were affected. This showed that variable kinetics applies for acetate utilization, depending on the culture history and feeding regime. Biros et al. [22] investigated the effect of variations in the acetate to biomass ratio on substrate storage potential and on the kinetics of substrate utilization. They found that biomass adapted to acetate content fluctuations by adjusting its metabolic reaction rates: lower acetate to biomass ratios diverted a larger substrate fraction to storage, high acetate levels increased the substrate fraction directly utilized for growth, and high acetate levels increased growth rate whereas low ones enhanced the storage rate. On the other hand, acetate has proved to be a useful pattern substrate for stoichiometric parameter estimation, and has been used by a number of authors.

The stoichiometric parameter values obtained for acetate were adequate for the hospital wastewater degradation simulation. When the experimental OUR profile is analysed, some points can be observed to differ from the functioning with acetate as substrate: 1 - the storage rate of substrate is higher than the growth rate based on S_B, 2 - the growth based on X_{OHO_stor} rapidly becomes higher than the other phenomena, 3 - XC_B is responsible for the majority of the COD most of the time. These remarks are consistent with the nature of the two substrates and with the kinetic parameters evaluated for these reactions.

It can also be seen that the second peak is higher than the first one. In this case the model was not capable of simulating this phenomenon. It seems as if the microorganisms "wake up" with a first pulse after a famine phase and, when a second pulse arrives, they are in better condition and ready for substrate degradation, showing higher activity. This phenomenon was also observed by Sin et al. [41] and Fan et al. [29], who attributed it to the improved ability of biomass to sustain a higher growth rate after the first pulse of acetate. The same phenomenon was observed by Delgado et al. [44], even with the addition of a cocktail of PhACs between the two acetate pulses. Vanrolleghem et al. [45] and Guisasola et al. [28] added a first pulse of acetate to induce a "wake-up" effect on the biomass activity.

It may be pointed out that, in this step of the study (respirometric trials with hospital wastewater), the concentration of substrate and biomass inside the biological reactor was reduced after each pulse, as result of the addition volume of hospital wastewater volume addition (200 mL for each pulse), which could be expected to lead a corresponding reduction of biomass respiration rate. Nevertheless, according to experimental OUR data, it seems that, during the feast period of each degradation pulse, the effect of volume variations on the biomass were almost negligible, while at the end of the OUR profile (famine period) the influence of volume on biomass activity was more perceptible (Fig. 9). The fact that the volume variation effect on biomass activity had been negligible during the feast phase can be attributed to the biomass "wake up" effect explained above. When acetate is used as carbon source, the volume variation does not have a significant influence on the biomass and biodegradable substrate concentrations, because small volumes of concentrated acetate are generally used.

With all these remarks, it is important to note the good correspondence between the experimental data and the model outputs for calibration and validation processes, which demonstrates the feasibility of the calibration process and of the COD fractionation method adopted in this study.

The experimental OUR profiles obtained in this study prove that microorganisms , as has already been demonstrated in [9,10,13,14], they adapt to the specificity of a hospital wastewater.

Table 6
Numerical results of calibration and validation processes using filtered hospital wastewater as carbon source

No.	Type	Symbol	Range	Hospital wastewater
1	*Kinetic parameters*	q_{SB_Stor}, $gCODgCOD^{-1}$	1–15	12.39
2		$q_{XCB\_SB,hyd}$, $gCODgCOD^{-1}$	1–5	3
3		K_{XCB_hyd}, $gCODgCOD^{-1}$	0.1–8	0.21
4		$\mu_{OHO,Max}$, d^{-1}	1–15	2.03
5		K_{SB_OHO}, $gCODm^{-3}$	0.20–20	14.95
6		K_{Stor_OHO}, $gCODgCOD^{-1}$	0.001–1	0.046
7		K_{NHx_OHO}, gNm^{-3}	0.01–0.05	0.01
MRE of estimation of OUR for calibration (filtered wastewater) (329 mgCOD L^{-1}), %				4.18
MRE of estimation of OUR for validation (non-filtered wastewater) (564 mgCOD L^{-1}), %				6.24

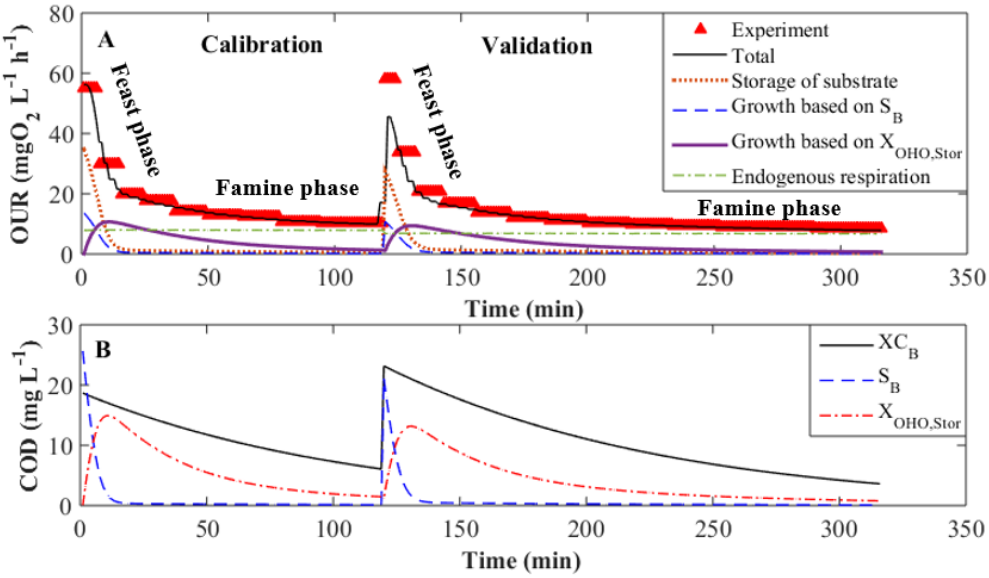

Fig. 9. Oxygen uptake rate (A) and substrate behaviour expressed as COD (B) for the individual processes corresponding to the calibration and validation processes for M1 theory, using filtered (First peak) and non-filtered (Second peak) hospital wastewater, respectively, as carbon source.

4. Conclusions

With the objective of determining a model that can represent the degradation of a hospital wastewater with an adapted biomass, the following three steps were carried out, leading to intermediate conclusions.

The stoichiometric and kinetic parameters were estimated and validated using acetate as carbon source according to two theories: the theory considering that microorganisms use the carbon reserve and easily biodegradable substrates simultaneously for growth was the most adequate for predicting the experimental OUR profile.

The adaptation of a procedure for the estimation of easily and slowly biodegradable substrates considering simultaneous growth and substrate storage was successfully validated. The high value obtained for undegradable soluble and particulate matter is consistent with the hospital wastewater effluent (PhACs, powerful cleaners, etc.).

The more suitable model was used for first time in the simulation of a hospital wastewater degradation process, and the values obtained for the kinetic parameters were different from those obtained for the same system using acetate as carbon source. This proves the influence of substrate nature, at least in the system studied. Oxygen uptake rates were able to calibrate the kinetic coefficients for hospital wastewater treatment. The modification of the stoichiometric and the kinetic parameters of the model concerning the phenomena that take place during the degradation of easily biodegradable substrate, with a higher proportion of the storage mechanism, showed an adaptation of the microorganism to the hospital wastewater.

The approach developed in this paper shows the necessity to adapt an ASM model to the specificity of hospital wastewater treatment systems. Drawing on this, ASM models can be used for simulating the hospital wastewater degradation. The stoichiometric and kinetic parameters should be calibrated using the real feeding substrate. In the near future, this model will be included in a full SMBR model and tested with a functioning pilot.

Symbols

ΔO_{2_SB} — Oxygen consumption for S_B degradation ($mgO_2\,L^{-1}$)

ΔO_{2_XCB} — Oxygen consumption for XC_B degradation ($mgO_2\,L^{-1}$)

μ_{OHO_Max} — Maximum growth rate of heterotrophic microorganisms (d^{-1})

ASM — Activated sludge model

ATU — Allylthiourea

COD_T — Total chemical oxygen demand ($mg\,L^{-1}$)

f_{STO} — Stored fraction of easily biodegradable substrate ($gCOD\,gCOD^{-1}$)

$f_{SU\_XCB,hyd}$ — Production rate of S_U in hydrolysis of X_{CB} ($gCOD\,gCOD^{-1}$)

$f_{XU\_Bio,lys}$ — Fraction of undegradable particulate matter, X_U, generated in biomass decay, X_{OHO} ($gCOD\,gCOD^{-1}$)

i_{N_SB} — Nitrogen content in easily biodegradable substrate, S_B ($gNg\,COD^{-1}$)

i_{N_SU} — Nitrogen content in undegradable substrate, S_U ($gNg\,COD^{-1}$)

i_{N_XBio} — Nitrogen content of biomass ($gNg\,COD^{-1}$)

i_{N_XCB} — Nitrogen content in biodegradable particulate matter, XC_B ($gNg\,COD^{-1}$)

i_{N_XU} — Nitrogen content of undegradable particulate matter, X_U ($gNg\,COD^{-1}$)

K_{NHx_OHO} — Half-saturation coefficient for ammonium ($gN\,m^{-3}$)

K_{O2_OHO} — Half-saturation coefficient for oxygen ($gO_2\,m^{-3}$)

K_{SB_OHO} — Half-saturation coefficient for easily biodegradable substrate ($gCOD\,m^{-3}$)

K_{Stor_OHO} — Half-saturation coefficient for the ratio of stored material/heterotrophic microorganisms (gCOD $gCOD^{-1}$)
K_{XCB_hyd} — Hydrolysis saturation constant (gCOD $gCOD^{-1}$)
$m_{OHO,Ox}$ — Biomass endogenous decay rate coefficient (d^{-1})
$m_{Stor,Ox}$ — Endogenous decay rate coefficient of stored material (d^{-1})
$OC_{G,SB}$ — Oxygen consumption for growth based on S_B ($mgO_2\,L^{-1}$)
$OC_{G,stor}$ — Oxygen consumption for growth based on stored material ($mgO_2\,L^{-1}$)
OC_{Stor} — Oxygen consumption for storage ($mgO_2\,L^{-1}$)
OUR — Oxygen uptake rate ($mgO_2\,L^{-1}\,h^{-1}$)
OUR_{end} — Endogenous oxygen uptake rate ($mgO_2\,L^{-1}\,h^{-1}$)
OUR_{exp} — Experimental oxygen uptake rate ($mgO_2\,L^{-1}\,h^{-1}$)
OUR_{model} — Model oxygen uptake rate ($mgO_2\,L^{-1}\,h^{-1}$)
q_{SB_Stor} — Rate constant for stored storage of easily biodegradable substrate (gCODg $COD^{-1}d^{-1}$)
$q_{XCB\_SB,hyd}$ — Hydrolysis rate constant (gCOD $gCOD^{-1}\,d^{-1}$)
MRE — Mean relative error
S_B — Easily biodegradable substrate concentration (gCOD m^{-3})
$SGSS$ — Simultaneous growth and substrate storage
S_{NHx} — Ammonium concentration (g Nm^{-3})
S_{O2} — Dissolved oxygen concentration ($gO_2\,m^{-3}$)
S_U — Undegradable soluble substrate concentration (gCOD m^{-3})
V_{sludge} — Sludge volume (L)
V_w — Wastewater volume (L)
$WWTP$ — Wastewater treatment plant
XC_B — Biodegradable particulate matter concentration (gCOD m^{-3})
X_{OHO} — Heterotrophic microorganism concentration (gCOD m^{-3})
$X_{OHO,Stor}$ — Stored material concentration (gCOD m^{-3})
X_U — Undegradable particulate matter concentration (gCOD m^{-3})
$Y_{SB\_OHO,Ox}$ — Yield for heterotrophic microorganism growth per easily biodegradable substrate (Aerobic) (gCOD $gCOD^{-1}$)
$Y_{SB\_Stor,Ox}$ — Yield for stored material formation per easily biodegradable substrate (Aerobic) (gCOD $gCOD^{-1}$)
$Y_{Stor\_OHO,Ox}$ — Yield for heterotrophic microorganism growth per stored material (Aerobic) (gCOD $gCOD^{-1}$)
$Y_{SB\_OHO,Ox,net}$ — Net heterotrophic yield (gCOD $gCOD^{-1}$)

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