An off-line respirometric procedure to determine inhibition and toxicity of biodegradable compounds in biomass from an industrial WWTP

A. Guisasola*, J.A. Baeza, J. Carrera, C. Casas and J. Lafuente

Chemical Engineering Dept., Universitat Autònoma de Barcelona, Bellaterra 08193, Spain

* Corresponding author (E-mail: *Albert.Guisasola@uab.es*)

Abstract Determining whether a certain compound is toxic (or inhibitor) or not to a biomass of a WWTP is crucial in order to avoid WWTP malfunction. Respirometric techniques have been commonly used to assess the toxicity of a certain compound by evaluating the oxygen uptake rate (OUR) profile obtained when a pulse of substrate is added to endogenous biomass and comparing it with the OUR profile obtained when the pulse is a mixture of substrate and the possible toxic or inhibitor (PTI). However, when using this method with biodegradable compounds some difficulties arise because the PTI consumption implies oxygen consumption as well. In this study, a modified procedure for toxicity assessment using respirometry is developed which overcomes the difficulties caused by the toxic biodegradability. This procedure is based on the comparison of different respirograms obtained with pulses of control substrate before and after adding the PTI and with a pulse of PTI and control substrate together. Moreover, some examples are shown with p-phenylenediamine and phenol as an example of toxic and inhibitor for nitrifying biomass.

Keywords Inhibition; modelling; nitrifying biomass; oxygen uptake rate; respirometry; toxicity

Introduction

Biomass inhibition or death is a huge problem for WWTPs because recovering or renewing biomass can be very complex and lengthy. Hence, determining whether a compound is toxic or not before it gets in touch with the WWTP biomass becomes crucial. This feature is even more important when dealing with industrial WWTPs where the influent can be very changeable in load and composition and the possibility of a toxic presence in the influent is higher than in conventional WWTPs.

A popular respirometric technique to assess the toxicity of a certain compound consists of evaluating the slope value in the dissolved oxygen (DO) profile obtained after adding a substrate pulse and comparing it with the slope value obtained when the pulse is a mixture of substrate and possible toxic (Volskay and Grady, 1990) (Figure 1b). The BIOMATH group (Ghent University) have proposed a different method for toxicity assessment based on respirograms comparison using a commercial respirometer named RODTOX (Kong *et al*., 1996) (Figure 1a).

However, when using these two methods to determine the toxicity of biodegradable compounds some difficulties arise because the toxic consumption implies oxygen consumption as well. Then, respirograms obtained adding the substrate plus the possible toxic or inhibitor (PTI) cannot be reliably compared to the ones obtained adding only substrate. Indeed, it can be really complex to distinguish between the oxygen consumption due to substrate and the oxygen consumption due to the PTI, especially when biodegradability rates of PTI and substrate are similar.

In this study, a modified procedure of the one proposed by the BIOMATH group with an aerated respirometer is developed. This modified procedure overcomes the difficulties due to the toxic biodegradability by characterising the toxic consumption and the OUR profile obtained during this consumption.

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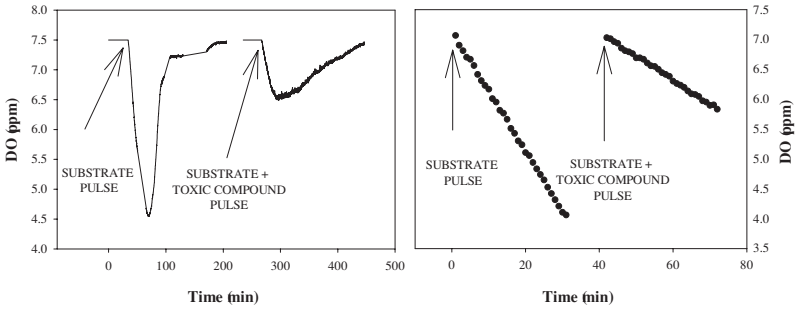


Figure 1 Toxicity assessment a) with an aerated respirometer and b) comparing the slope value of the DO profile

Methods

Respirometer experimental set-up

In this study, a LFS respirometer has been used (Figure 2). In this respirometer, the airflow is continuous and constant and the DO is measured in the liquid phase, which has no inputs or outputs.

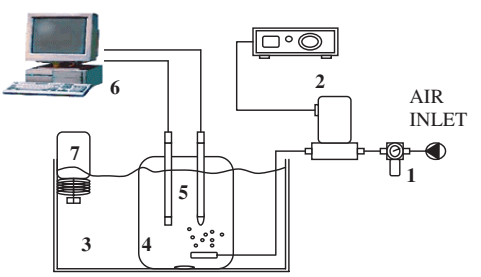
The air inlet goes through a manometer and then through an airflow meter (Brooks 825) in order to ensure that the airflow is constant. Then, the air is diffused to the respiration vessel of 1 L through an air diffuser. The respiration is magnetically stirred and pH, DO and temperature are measured in the liquid phase through the DO probe (WTW-Cellox 325) and the pH probe (WTW-Sentix 81), which are connected via RS-232 with the PC, which stores and monitors the data transmitted by the probes. The respiration vessel is submerged in an isothermal bath to maintain the temperature at 31°C. The pH in the respiration vessel is maintained in the optimum pH range for nitrification.

LFS respirometer principle

A typical respirogram obtained with the LFS respirometer is shown in Figure 3. The respirogram starts with the aeration stopped in order to calculate the value of the endogenous OUR (OUR_{END}), which is equal to the slope value of the DO profile. Then, the vessel is aerated again and the DO level increases until it reaches a constant value called SO_E. This level balances the external oxygen transfer due to aeration with the OUR_{END}. The value of the global oxygen transfer coefficient (k_La) must be estimated for OUR profile calculation. The k_La of the system is estimated through a non-linear least square optimisation with the reaeration profile according to Eq. (1):

$$\frac{dSo}{dt} = k_La \cdot \left[SO_E - S_O(t)\right] \quad (1)$$

Then, a pulse of substrate is added and $S_O(t)$ decreases because of the OUR due to the exter-



1 - manometer
2 - airflow meter
3 - isothermal bath
4 - magnetic stirrer and air diffuser
5 - pH and DO probes
6 - PC
7 - thermostat

Figure 2 Experimental set of the LFS respirometer

nal substrate (exogenous OUR or OUR_{EX}). The mathematical expression that describes this situation is:

$$\frac{dSo}{dt} = k_L a \cdot \left[SO_E - S_O(t)\right] - OUR_{EX} \quad (2)$$

Then, when the substrate is totally consumed, the DO level returns again to the SO_E level, because at this moment the OUR value is equal again to OUR_{END}. This procedure can be considered as simple because it only requires DO measures, an ON/OFF valve to control the aeration and a pH control (when substrates consumption affects the pH). The airflow value is not needed, but it must be constant. The OUR profile is obtained mathematically form the LFS respirogram by solving the oxygen balance in the liquid phase in each point of the respirogram (Eq. (2)).

Chemical analyses

Total Suspended Solids (TSS), Volatile Suspended Solids (VSS) and ammonium were done according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). Nitrates and nitrites were measured by capillary electrophoresis using a WATERS Quanta 4000E CE with a commercial solution of WATERS as electrolyte.

Results and discussion

Parameters studied

The parameters used in this study to compare respirograms are obtained from either the respirogram (DO profile) or the OUR profile. These parameters are shown on Figure 3 and described in Table 1. New software has been programmed in MATLAB® 6.1 (The MathWorks, Natick, MA), which calculates the OUR profile from Eq. (2) and also all the parameters described next.

Moreover, a model has been fitted to the OUR profile to determine the biokinetic parameters of the biomass. This model is based on the ASM1 model (Henze *et al.*, 2000) and includes both autotrophic and heterotrophic processes. However, in the model presented in Table 2 there are only included processes related to autotrophic biomass (X_{AU}) growth, because the examples used in this study are focused on assessing toxicity and inhibition on nitrifying biomass.

In this study, the nitrite oxidation is slower than the ammonium oxidation and, then, nitrite accumulates and a two-step nitrification model can be fitted. The second oxygen consumption observed during the ammonium pulse consumption is due to the nitrite oxidation. Nitrite was measured in some runs and this assumption was demonstrated (data not shown).

Toxicity and inhibition determination procedure

The procedure proposed in this study determines the inhibition or toxicity of a certain wastewater, even when it has a biodegradable fraction. A control substrate is needed, so

Table 1 Description of the parameters studied

Parameter	Unit	Profile source	Description
OUR_{END}	$g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$	DO	The specific slope from the DO profile with no aeration
PS	$g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$	DO	The initial specific slope from the DO profile after the pulse
PA	$mg\ O_2 \cdot min \cdot L^{-1}$	DO	The area between the SO_E level and $S_O(t)$
PH	$mg\ O_2 \cdot L^{-1}$	DO	The maximum difference between the SO_E and $S_O(t)$
OUR_{MAX}	$g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$	OUR	The maximum value of the specific OUR profile
CT	min	OUR	The time spent to consume the external substrate
OC	$mg\ O_2 \cdot L^{-1}$	OUR	The total oxygen consumed: area under the OUR profile

Table 2 Two step nitrification model: 1-ammonia-oxidisers, 2-nitrite-oxidisers, f_i (active fraction), Y_i (biomass yield; $Y_1 = Y_2 = 0.24$ g $COD_X \cdot gN^{-1*}$), μ_i (specific growth rate), K_{Si} (semisaturation constant), $K_O = 0.2$ mg $O_2 \cdot L^{-1}$ (oxygen semisaturation constant;), K_t (time constant introduced to describe the delay observed in reaching the OUR_{MAX} of the OUR profile). This first order delay has also been suggested by Vanrolleghem *et al*. (1998)

* values taken from the literature (Henze *et al*., 2000)

Process	S_{NH_4}	S_{NO_2}	S_{NO_3}	S_O	X_{AU}	Kinetics
Growth on ammonia	$-\frac{1}{Y_1}$	$\frac{1}{Y_1}$		$-\frac{(3.43-Y_1)}{Y_1}$	1	$\mu_{1MAX} \cdot f_1 \frac{[N-NH_4^+]}{K_{S1}+[N-NH_4^+]} \cdot \frac{[DO]}{K_O+[DO]} \cdot e^{(1-\frac{t}{K_t})} \cdot X$
Growth on nitrite		$-\frac{1}{Y_2}$	$\frac{1}{Y_2}$	$-\frac{(1.14-Y_2)}{Y_2}$	1	$\mu_{2MAX} \cdot f_2 \frac{[N-NO_2^-]}{K_{S2}+[N-NO_2^-]} \cdot \frac{[DO]}{K_O+[DO]} \cdot X$

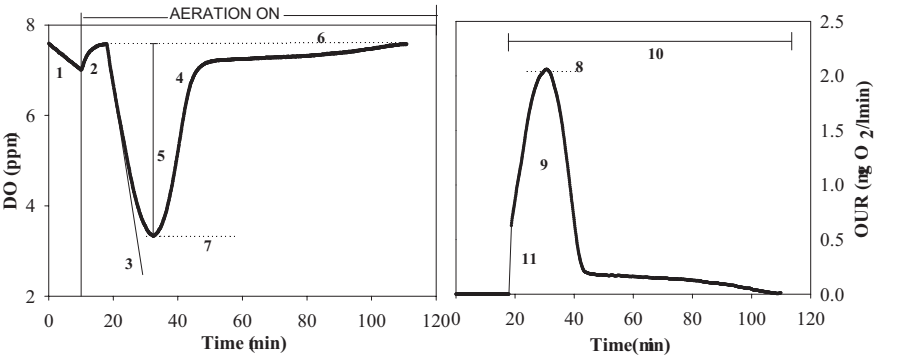


Figure 3 A typical LFS respirogram (a) and the OUR profile (b) obtained with 10 mg $N\text{-}NH4^+ \cdot L^{-1}$: 1 – OUR_{END}, 2 – Reaeration profile, 3 – Initial peak slope (PS), 4 – Peak Area (PA), 5 – Peak Height (PH), 6 – SO_E, 7 – SO_{MIN}, 8 – OUR_{MAX}, 9 – Oxygen Consumed (OC), 10 – Consuming time (CT), 11 – OUR model

that the effect of the possible toxic or inhibitor (PTI) can be assessed. Depending on which control substrate is used (ammonium nitrogen or organic substrate) the effect on the autotrophic or heterotrophic biomass will be observed. If the substrate control is a mixture, the global effect will be observed.

Four runs are needed to state the PTI inhibition or toxicity level. In this study, toxicity is regarded as the negative effect observed on biomass when the PTI has already been consumed (at least its biodegradable fraction). On the other hand, inhibition is regarded as the negative effect observed on biomass when the control substrate and the PTI are added together. However, different combinations of toxicity and inhibitory effects can be found depending on the PTI used and the concentration of the PTI tested. The complete procedure is next summarised and then, three graphical examples are shown.

1. The first run is obtained by the addition of a control substrate pulse. The initial aeration stop and the posterior reaeration profile will be used to calculate the OUR_{END} and k_La values.
2. In the second run, after the control substrate is consumed, a PTI pulse is added.
3. The third run is obtained with the addition of the same pulse of control substrate added in the first one. The toxicity level of PTI can be assessed by comparing the first and the third respirograms. It is necessary to remark that the endogenous level (SO_E) must be reached after the PTI pulse to ensure that the biodegradable fraction of the PTI has totally been consumed. Toxicity can be quantified with the parameters described above according to Eq. (3).

$$\% \text{ toxicity} = \left(\frac{\text{PARAMETER run 1} - \text{PARAMETER run 3}}{\text{PARAMETER run 1}} \right) \cdot 100 \quad (3)$$

4. Then, biomass in the respirometer is changed for another biomass, which has been maintained in the same conditions as the starting biomass in the first run. This change is carried out in order to avoid possible interference because of the PTI added in run 3. Run 4 is obtained with the addition of together the substrate and PTI pulse. The comparison between run 4 and run 1 of each experiment is carried out for inhibition assessment. This comparison is done after having subtracted the parameters due to toxic consumption (run 2) from the parameters of run 4. The inhibition assessment is carried out only with the parameters related to the OUR profile according to Eq. (4), because the parameters of the respirogram are not strictly additive.

$$\% \text{ inhibition} = \left(\frac{\text{PARAMETER run 1} - (\text{PARAMETER run 4} - \text{PARAMETER run 2})}{\text{PARAMETER run 1}} \right) \cdot 100 \quad (4)$$

In the examples presented, the OUR_{END} is very low, probably because the biomass used was mainly nitrifying. Then, the oxygen transfer due to the surface contact between the liquid and the atmosphere when calculating OUR_{END} is not negligible and the OUR_{END} cannot be used for toxicity assessment.

Example A: PTI = low biodegradable toxic

The substrate control utilised in this experiment is ammonium chloride and the PTI used is *p*-phenylenediamine (5 mg·L^{-1}). The biomass comes from a pilot plant where nitrifying microorganisms are strongly favoured. The graphical results are shown in Figure 4 and in Table 3.

Example B: PTI = biodegradable toxic

The substrate control utilised in this experiment is ammonium chloride and the PTI used is *p*-phenylenediamine (2.5 mg·L^{-1}) plus acetate (20 mg COD·L^{-1}). This synthetic PTI is utilised in order to emulate the effect of a wastewater that contains biodegradable and toxic compounds. The biomass comes from the same pilot plant of the previous example. The graphical results are shown in Figure 5 and in Table 4.

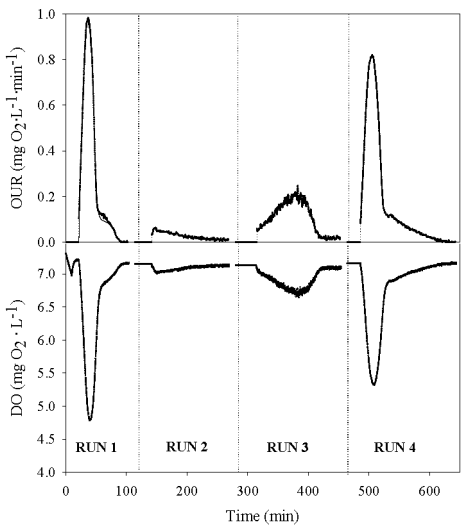


Figure 4 DO and OUR profiles obtained in example A

Table 3 Parameters calculated in example A

Run number	A.1	A.2	A.3	A.4
Substrate pulse	N	PIT	N	N+PIT
$mg \cdot L^{-1}$	5	5	5	5 + 5
OUR_{END} ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.055	0.055	0.059	0.058
PS ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.344	0.033	0.014	0.232
PA ($mg\ O_2 \cdot min \cdot L^{-1}$)	56.5	8.1	29.4	51.4
PH ($g\ O_2 \cdot L^{-1}$)	2.37	0.14	0.45	1.84
OUR_{MAX} ($gO_2 \cdot gVSS^{-1} \cdot min^{-1}$)	1.89	0.12	0.40	1.58
OC ($mg\ O_2 \cdot L^{-1}$)	22.7	3.4	11.9	26.9
CT (min)	70	105	116	141
X ($mg\ VSS^{-1} \cdot L^{-1}$)	750	750	750	750
$\mu_{1MAX} \cdot f_1 \cdot 10^5$ (min^{-1})	13.29			8.375
Ks_1 ($mg\ N\text{-}NH_4^+ \cdot L^{-1}$)	1.145			0.924
$\mu_{2MAX} \cdot f_2 \cdot 10^5$ (min^{-1})	2.750			2.385
Ks_2 ($mg\ N\text{-}NO_2^- \cdot L^{-1}$)	0.322			1.270
K_t (min)	13.8			13.8

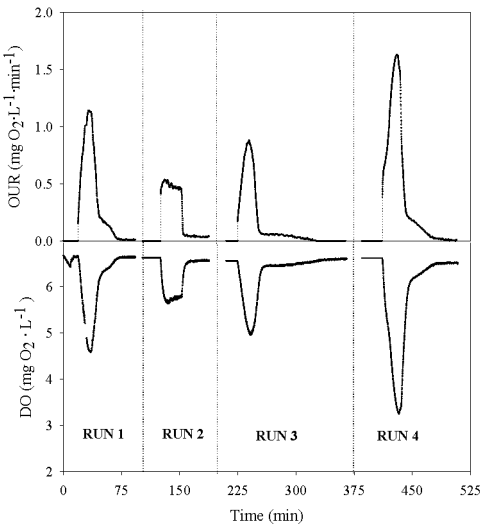


Figure 5 DO and OUR profiles obtained in example B

Table 4 Parameters calculated in example B

Run number	B.1	B.2	B.3	B.4
Substrate pulse	N	PIT	N	N+PIT
$mg \cdot L^{-1}$	5	2.5+20	5	5+2.5+20
OUR_{END} ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.040	0.042	0.040	0.045
PS ($mg\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.260	0.374	0.181	0.421
PA ($mg\ O_2 \cdot min \cdot L^{-1}$)	41.0	26.7	34.3	77.0
PH ($mg\ O_2 \cdot L^{-1}$)	2.07	0.97	1.58	3.33
OUR_{MAX} ($g\ O_2 \cdot g\ VSS^{-1} \cdot min^{-1}$)	1.73	0.81	1.33	2.47
OC ($mg\ O_2 \cdot L^{-1}$)	22.2	14.3	19.0	36.9
CT (min)	73	30	96	52
X ($mg\ VSS^{-1} \cdot L^{-1}$)	950	950	950	950
$\mu_{1MAX} \cdot f_1 \cdot 10^5$ (min^{-1})	12.3		5.30	13.6
Ks_1 ($mg\ N\text{-}NH_4^+ \cdot L^{-1}$)	1.54		0.13	1.50
$\mu_{2MAX} \cdot f_2 \cdot 10^5$ (min^{-1})	3.35		2.03	3.51
Ks_2 ($mg\ N\text{-}NO_2^- \cdot L^{-1}$)	1.04		1.37	1.05
K_t (min)	9.23		5.93	9.23

Discussion of examples A and B

The numerical comparison of the parameters obtained in run 3 and run 1 of each example shows a considerable toxicity level. In example A, the toxicity levels obtained with the parameters related to the DO profile (PS, PA, PH) and the parameters related to the OUR profile (OC and OUR_{MAX}) range from 75 and 90% in most of the parameters. On the other hand, in example B the range decreases to 15–30% because the PTI concentration is lower. The parameters related to the oxygen consumption rate (PS, PH and OUR_{MAX}) are the most sensitive to toxicity (mainly the PS, which shows the highest toxicity level in all the cases). On the other hand, the parameters related to the total oxygen consumption (PA and OC) give lower toxicity values. As seen on both tables, another good toxicity indicator is the time necessary to consume the control substrate. The comparison between the parameters of the model fitted to the OUR profile could give an idea of how the PTI affects the biomass. In example B, the toxic effect is observed because both maximum growth rates (μ_{1max} and μ_{2max}) decrease considerably. In experiment A, the model used cannot be fitted to the experimental data of run 3.

In experiment A, an important toxic effect can be observed but not an inhibitory effect, although the same amount of PTI is added in runs 3 and 4. This PTI requires a certain time to have a negative effect on the biomass. The inhibitory levels obtained from parameter comparison between runs 1 and 4 ranges from 20 to 45% in example A and from 0 to 5% in example B. Moreover, the oxygen consumed in run 4 of both experiments coincides with the sum of the oxygen consumed in run 1 and run 2. This means that the whole control substrate pulse is consumed despite the PTI presence. The OUR model is fitted to the new OUR profile obtained subtracting the OUR profile of run 2 from the OUR profile of run 4. Then, the parameters of the model obtained are compared to the parameters of the model fitted to run 1. The PTI works mainly by decreasing the μ_{1max} value and increasing the value of the K_{S2}. Both are typical inhibitory effects upon the biomass.

Two types of inhibition are generally considered: competitive and non-competitive. In the competitive inhibition, the substrate and the inhibitor compete for the same enzymatic site. When modelling this effect the semisaturation constant increases due to the inhibitor (Orhon and Artan, 1994). This effect is observed in the nitrite-oxidising bacteria. On the other hand, in the non-competitive inhibition the substrate is unable to prevent the enzyme-inhibitor combination. The mathematical effect observed is the decrease of maximum growth rate (Orhon and Artan, 1994). This effect is observed in the ammonium-oxidising bacteria.

If experiment A was carried out with the other respirometric procedures found in the literature the inhibition effect would be observed, but this effect would be underestimated because the oxygen consumption due to the PTI wouldn't be subtracted. In experiment B, the inhibitory effect could not be assessed because the oxygen consumption due to the PTI cannot be omitted at all. Then, the OUR profile due to the PTI consumption would mask the OUR profile due to the control substrate pulse. Moreover, the toxic effect could not reliably be assessed. In the case where the toxic needs a certain time to have effect, the conventional methods are not directly applicable.

Example C: PTI = low biodegradable inhibitor

The substrate control utilised in this experiment is ammonium chloride and the PTI used is phenol (5 $mg \cdot L^{-1}$). The biomass used in this experiment is a mixture of a mainly nitrifying biomass and heterotrophic biomass. The literature indicates that concentrations of phenol near 5.8 $mg \cdot L^{-1}$ inhibit nitrification 75% (Randall *et al.*, 1992). The results are shown in Figure 6 and in Table 5.

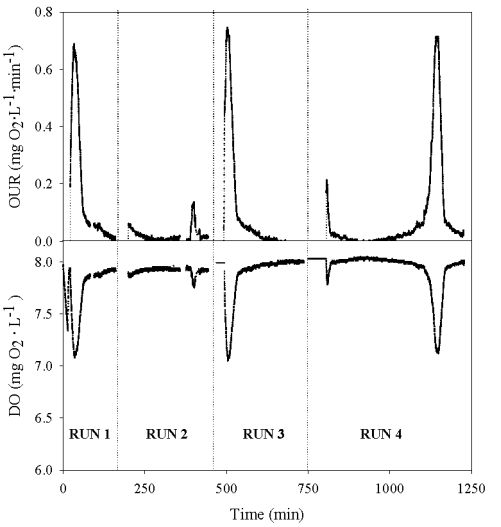


Figure 6 DO and OUR profiles obtained in experiment C

Table 5 Parameters calculated in example C

Run number	C.1	C.2	C.3	C.4
Substrate pulse	N	PTI	N	N+PTI
$mg \cdot L^{-1}$	5	5	5	5 + 5
OUR_{END} ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.05	0.11	0.05	0.06
PS ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.103	0.002	0.152	0.088
PA ($mg\ O_2 \cdot min \cdot L^{-1}$)	27.6	5.7	26.8	38.0
PH ($gO_2 \cdot L^{-1}$)	0.84	0.17	0.92	0.89
OUR_{MAX} ($g\ O_2 \cdot gVSS^{-1} \cdot min^{-1}$)	0.80	0.16	0.86	0.82
OC ($mg\ O_2 \cdot L^{-1}$)	22.3	4.9	22.0	30.9
CT (min)	138	243	132	475
X ($mg\ VSS^{-1} \cdot L^{-1}$)	1250.0	1250.0	1250.0	1250.0
$\mu_{1MAX} \cdot f_1 \cdot 10^5$ (min^{-1})	3.63		3.93	
Ks_1 ($mg\ N\text{-}NH_4^+ \cdot L^{-1}$)	1.04		1.04	
$\mu_{2MAX} \cdot f_2 \cdot 10^5$ (min^{-1})	0.85		1.62	
Ks_2 ($mg\ N\text{-}NO_2^- \cdot L^{-1}$)	0.45		1.74	
K_t (min)	4.1		3.4	

Apparently, graphical run 3 is very similar to graphical run 1 and, then, no toxic effect can be observed. Moreover, when comparing the parameters of run 3 and run 1 no toxic effect can be observed because the parameters are quite similar, even the consumption time parameter. Both runs are so similar because the ammonia-oxidisers are not affected by the PTI and in the OUR profile the oxygen consumed due to ammonia oxidation is predominant. However, it can be observed that the way that nitrite-oxidisers consume oxygen has changed after having added the PTI. The total oxygen consumed in both runs is the same, but the OUR profile due to nitrite oxidation has changed. This change is expressed in the model by an increase in the μ_{2max} and in the K_{S2}.

On the other hand, when analysing run 4, an inhibitory effect due to the PTI can be observed. At the start of run 4, there is a little oxygen consumption due to PTI consumption nor probably due to ammonia consumption. Again, the PTI seems to require a certain time to have an effect on the biomass. Once this first oxygen consumption is finished, there is a period where no oxygen is consumed at all. This period lasts approximately four hours, which practically coincides with the time required for the toxic consumption in run 2. Then,

after the PTI is consumed, the ammonia uptake starts again. Accordingly, this PTI is clearly an inhibitor for the nitrifying biomass used in this study. Moreover, the OUR_{MAX} of run 1, run 3 and run 4 are very similar. This fact indicates that, once the PTI is consumed, the ammonia consumption rate doesn't decrease. The OUR model proposed cannot be fitted to the OUR profile of run 4. A new model more complex is required to be fitted reliably to the OUR profile, which should take into account the PTI profile.

If experiment C was carried out with the procedure found in the literature it could not have been clearly demonstrated that, despite its strong inhibitory effect, the phenol is not toxic for the nitrifying biomass used in this experiment.

Conclusions

- An off-line respirometric procedure is presented which is able to give more information about toxicity and inhibition of biodegradable compounds than other techniques found in the literature.
- The parameters related to the oxygen consumption rate (PS, PH and OURMAX) and the time required to consume the control substrate are good indicators of a toxic presence. The parameters related to the oxygen consumption (PA and OC) indicate if the toxic presence has modified whether the whole substrate is consumed or not. Finally, the biokinetic parameters obtained fitting the OUR profile to a model based on ASM1 can give an intuition on how the PTI affects the biomass.
- The procedure described has been used successfully for the toxicity assessment of *p*-phenylendiamine. This toxic has the particularity that it requires a certain time to have an effect on the biomass. The procedure described has also been used to analyse the inhibitory and toxic effects of phenol on nitrifying biomass.

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