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SIMULTANEOUS DETERMINATION OF INHIBITION KINETICS OF CARBON OXIDATION AND NITRIFICATION WITH A RESPIROMETER

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Abstract—This paper presents a new method for the simultaneous determination of inhibition kinetics of both carbon oxidation and nitrification with the RODTOX (Rapid Oxygen Demand and TOXicity tester) respirometric biosensor. The biokinetic parameters of carbon oxidation and nitrification are estimated on the basis of respirometric data obtained from batch experiments with pulse injection of a mixture of a readily biodegradable carbon (acetic acid) and nitrogen (ammonium chloride) source. This estimation is based on a Double Monod mathematical model and a nonlinear parameter estimation algorithm. Performing batch tests with six to eight different concentrations of a toxic compound allows the deduction of the dependence of the kinetic parameters on the toxicant concentrations. The time necessary for a complete determination of the inhibition kinetics is approx. 8 h. Practical applications of the developed method are given.

Key words—activated sludge, biodegradation, biosensor, inhibition, model identification, oxygen uptake rate, respirometry, toxicity.

INTRODUCTION

Respirometric techniques have been intensively used for the determination of BOD (Köhne, 1985; Ros, 1993; Spanjers *et al.*, 1993; Vanrolleghem *et al.*, 1994), toxicity (King and Dutka, 1986; Kilroy and Gray, 1992; Kong *et al.*, 1993; Ros, 1993; Kim *et al.*, 1994; Nirmalakhandan *et al.*, 1994 and Vanrolleghem *et al.*, 1994) and biokinetic parameters (Gaudy *et al.*, 1990a, b; Tabak *et al.*, 1990; Vanrolleghem, 1994) of toxic and nontoxic wastewaters.

A recent development is the use of this technique for the Respiration Inhibition Kinetics Analysis (RIKA) to quantify the toxic (or inhibitory) effect of xenobiotic compounds on the biogenic-carbon (C) removal in biological wastewater treatment systems (Volskay and Grady, 1990; Volskay *et al.*, 1990; Kong *et al.*, 1994). The RIKA-procedure was first developed by Volskay and Grady (1990). The central idea of the protocol is that the Monod kinetic parameters describing the biodegradation of a biogenic-C source (e.g. butyric acid) are measured in the presence of three inhibitory concentrations of a selected toxicant and a blank. This RIKA-procedure involves manual respiration tests in four parallel respirometers in which 19 pulses of different biogenic substrate concentrations are added consecutively. The ARIKA, a faster and Automated RIKA method to quantify the inhibitory effect of toxicants on the biodegradation of biogenic-C source (acetic acid), was developed by Kong *et al.* (1994) with a respirometric biosensor. The main progress with respect to the RIKA-method proposed by Volskay and Grady (1990) is that the ARIKA procedure reduces the number of experiments to four (instead of 4 × 19) and that the complete characterization of the toxic effect can be carried out within 3 h. These optimizations could be achieved because an oxygen uptake rate profile obtained from a batch experiment with this biosensor provides a degradation rate vs substrate concentration data-set that covers the whole substrate concentration range required by the RIKA-test (Kong *et al.*, 1994). Using a model-based approach coupled to a non-linear parameter estimation algorithm, the kinetic parameters of the biodegradation process can be obtained. The results of batch tests with different toxicant concentrations (usually 4) then establish the dependence of the kinetic parameters on the toxicant concentrations.

Recently, due to the more stringent effluent discharge standards, nitrogen removal as well as BOD removal has become obligatory for both municipal and industrial wastewater treatment plants. A number of wastewater treatment plants thus have to be upgraded to include nutrient removal (Kroiss *et al.*, 1992; Nowak and Svardal, 1993). Nitrification is the critical step in the nitrogen removal process. Some pilot investigations on the extension of activated sludge plants have shown that the inhibition of the nitrification processes occurs

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regularly (Nowak and Svardal, 1993). Therefore, it is important to quantify the effects of toxicants on nitrification as well as on carbon oxidation.

Nowak and Svardal (1993) developed a RIKA method for quantifying the inhibitory effect of toxicants on nitrification by means of a respirometric technique. In their method, the $\mu_{max,A}$ (μ_{max} for the autotrophic biomass) is obtained as follows. First, the total oxygen uptake rate (OUR_{TOT}) is measured by injection of 50 mg NH_4^+-N/l subsequently, the heterotrophic respiration rate (OUR_H) is obtained by adding ATU (allylthiourea, a selective inhibitor for nitrification). The autotrophic respiration rate (OUR_A) can then be calculated by subtracting OUR_H from OUR_{TOT}. Finally, the $\mu_{max,A}$ is calculated by an equation (Nowak and Svardal, 1993). The $K_{S,A}$ (half saturation constant for autotrophic biomass growth on NH_4^+-N) is determined in a conventional way, i.e. by measuring the OUR_A at six different levels of ammonia nitrogen ranging from 0 to 50 mg NH_4^+-N/l. Afterwards $K_{S,A}$ is calculated using a nonlinear regression.

However, up to now, the RIKA method is limited to the study of the effects of toxicants on a single biogenic carbon (C) or ammonia nitrogen (N) substrate at a time. This paper presents a new ARIKA method for simultaneous determination of the inhibitory effect of a toxicant on the degradation of multiple biogenic substrates (C and N) with the RODTOX respirometric biosensor within the time period of a working day.

MATERIALS AND METHODS

Chemicals tested. 3,5-Dichlorophenol (DCP), Cu^{2+} (as $CuSO_4$) and CN^- (as KCN) were used as the test model toxicants. They were of analytical grade.

Sludge source. The activated sludge was obtained from the aeration basin of the sewage treatment plant of the hospital "Maria Middelares", Gent, Belgium. For the experiments in which repeatability was studied concentrated sludge was collected and stored at 4°C for maximum 2 weeks before use.

Biogenic substrate. The biogenic-C and -N substrates used for measuring the heterotrophic and autotrophic respiration rate of the sludge were acetate (an equimolar solution of HAc/NaAc) and ammonia nitrogen (NH_4Cl). Acetate and ammonia have been chosen because these substrates are major components of domestic wastewater (Volskay and Grady, 1990) and give a well defined respirogram without the need for adaptation of the sludge.

Respirometric biosensor. The RODTOX biosensor (Kelma bvba, Niel, Belgium) is an open and automated respirometer, consisting of an aerated bioreactor, filled with 10 l of activated sludge. When the activated sludge is in the endogenous phase, i.e. when the dissolved oxygen (DO) is at the baseline concentration, a pulse of substrate is injected. As a result the activated sludge will show an increased exogenous oxygen uptake rate (OUR_{ex}) until the substrate is oxidized completely (which typically takes 30 min). The DO profile (respirogram) is recorded from which an OUR_{ex} profile can be calculated. A detailed description of the sensor can be found elsewhere (Vanrolleghem *et al.*, 1994; Kong *et al.*, 1994).

The operating conditions of the RODTOX were: pH, 7.2 ± 0.2; temperature, 25 ± 0.1°C and biomass concentration, 2.5 g VSS/l. The pH and temperature were controlled by means of a pH controller and heating element which were built in the RODTOX biosensor. The substrate dosage was expressed as mg per litre of mixed liquor in the RODTOX reactor.

RESULTS

Principle

First, the bioreactor of the biosensor is filled with 10 l of fresh activated sludge. While being thermostated, it is aerated until the baseline DO level (DO_{bl}, endogenous phase) is reached. Subsequently, a dose of the C–N-substrate mixture is injected. Due to this substrate pulse, microorganisms immediately increase their respiration rate, which causes a decrease in dissolved oxygen (DO). After the substrate is oxidized completely, the DO level increases and returns to its baseline level. Then the sensor is ready for the injection of a new pulse of substrate mixture. The described DO profile resulting from this pulse substrate addition is called a "respirogram" (Fig. 1). All respirographic data (sampling interval, 10 s) are collected on a PC with a data-acquisition program (Willems, 1991) for further data analysis.

The DO concentration in the bioreactor is determined by two competing processes, namely oxygen supply by continuous aeration and microbial oxygen uptake for exogenous respiration (Equation 1).

$$\frac{dDO}{dt} = Kla(DO_{bl} - DO) - OUR_{ex}. \qquad (1)$$

With

Kla: = oxygen mass transfer coefficient (min^{-1})
DObl = dissolved oxygen at the baseline level (mg/l)
OUR_{ex} = exogenous oxygen uptake rate (mg O_2/l · min)

Exogenous oxygen uptake rate (OUR_{ex}) data are central to respiration inhibition kinetics analysis. They can be obtained from Equation (2) if the oxygen mass transfer coefficient (Kla) can be estimated, all other variables being measured. The methods that have been developed to estimate the Kla from respirograms can be found elsewhere (Vanrolleghem, 1994). Plotting the calculated OUR_{ex} vs time typically results in a profile as that in Fig. 1.

$$OUR_{ex} = Kla(DO_{bl} - DO) - \frac{dDO}{dt}. \qquad (2)$$

The exogenous oxygen uptake rate curve reflects the kinetics of aerobic biodegradation of C and N substrates by heterotrophic and autotrophic microbes of the activated sludge. In most cases, these processes are independent and their oxygen uptake rates may

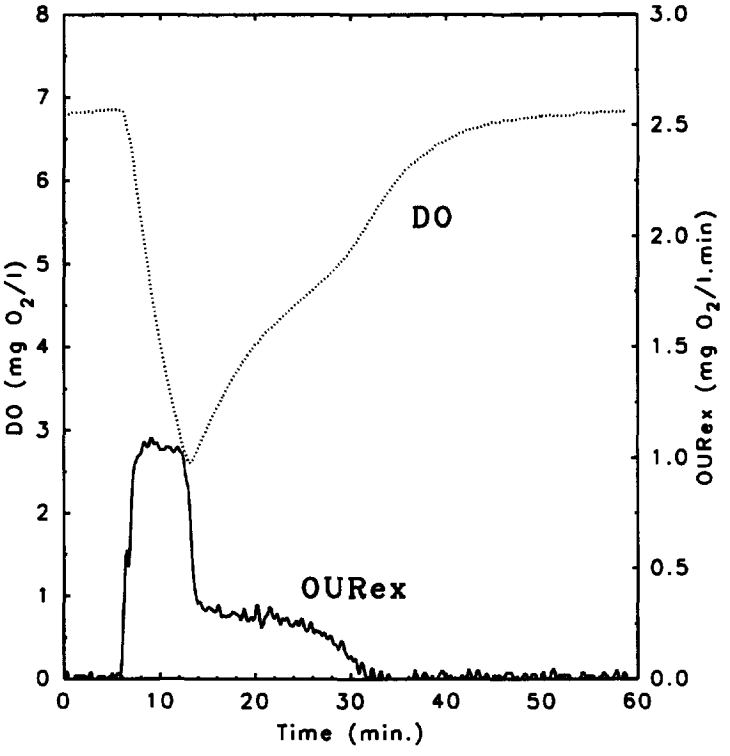

Fig. 1. A typical respirogram (dotted line) and corresponding exogenous respiration rate (OUR_{ex}, solid line) resulting from a pulse of C–N-substrate mixture.

be added up as indicated in Equation (3) and demonstrated further in Fig. 2.

$$OUR_{ex,TOT} = OUR_{ex,C} + OUR_{ex,N}. \quad (3)$$

Figure 2(a) shows the respirograms and corresponding exogenous respiration rates resulting from injection of respectively 20 mg COD, 2.5 mg N and a mixture of 20 mg COD + 2.5 mg N/l. A combined plot of the OUR_{ex} from Fig. 2(a) results in Figure 2(b). One clearly sees that the OUR_{ex} for the C–N-substrate mixture is the sum of OUR_{ex} for both C and N substrates injected separately.

Mathematical model

In a previous paper (Kong *et al.*, 1994) it was demonstrated that the OUR_{ex} profile for a single substrate contains the same type of information as the Monod growth curve in defining the relationship between growth rate (μ) and substrate concentration (S). This is because one batch experiment covers a range of substrate concentrations, making it possible to evaluate the "growth rate–substrate concentration" relationship. The degradation processes for the C–N-substrate mixture by activated sludge can be described by a Double Monod mathematical model as follows (Vanrolleghem and Verstraete, 1993)

$$r_A = -\frac{dS_{NH_4}}{dt} = \frac{\mu_{max,A} X_A}{Y_A} \frac{S_{NH_4}}{K_{S,A} + S_{NH_4}} \quad (4)$$

$$r_H = -\frac{dS_C}{dt} = \frac{\mu_{max,H} X_H}{Y_H} \frac{S_C}{K_{S,H} + S_C} \quad (5)$$

with

S_C, S_{NH_4} = carbon and NH_4 nitrogen substrate concentration (mgCOD/l or mgN/l)

X_H, X_A = heterotrophic and autotrophic biomass concentration (mgCOD/l)

$\mu_{max,H}$, $\mu_{max,A}$ = maximum specific growth rates for heterotrophic and autotrophic biomass (min^{-1})

$K_{S,H}$, $K_{S,A}$ = Monod half-saturation coefficient for carbon and NH_4 nitrogen substrates (mgCOD/l or mgN/l)

Y_H, Y_A = yield coefficients for heterotrophic and autotrophic biomass (mgCOD-biomassH/mgCOD or mgCOD-biomassA/mgN)

r_H, r_A = substrate degradation rates for heterotrophic (mgCOD/l · min) and autotrophic (mgN/l · min) biomass.

The substrate degradation rate r and substrate concentration S are related to the measured OUR_{ex} (Henze *et al.*, 1987)

$$OUR_{ex}(t) = r_H(1 - Y_H) + r_A(4.57 - Y_A)$$

$$= \frac{1 - Y_H}{Y_H} \frac{\mu_{max,H} S_C X_H}{K_{S,H} + S_C} + \frac{4.57 - Y_A}{Y_A} \frac{\mu_{max,A} S_{NH_4} X_A}{K_{S,A} + S_{NH_4}}. \quad (6)$$

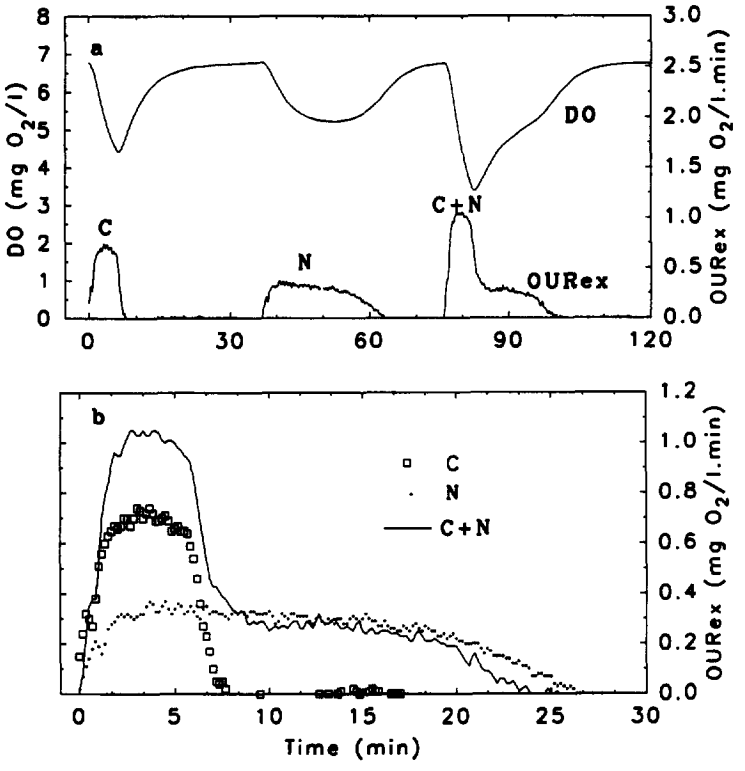

Fig. 2. Illustration of adding up heterotrophic and autotrophic respiration rates (OUR_{ex}) for biogenic C- and N-substrates. For more details, see text.

One should remark that the biomass growth due to the substrate addition is negligible within the time frame of the test. Indeed, addition of only 20 mg COD + 2.5 mg NH_4^+-N/l results in a biomass growth (approx. 10 mg/l) which is very small compared to the 2.5 g VSS/l already present in the mixed liquor.

Parameter estimation

It can be shown that, from the respiration rate only, not all parameters can be uniquely estimated (Dochain *et al.*, 1995). The parameters or parameter combinations that can be identified from Equation (6) are the combinations involving the initial substrate concentrations $(1 - Y_H)S_C$, $(4.57 - Y_A)S_{NH4}$, and the combinations involving the kinetic parameters $\mu_{max,H}(1 - Y_H)X_H/Y_H$, $\mu_{max,A}(4.57 - Y_A)X_A/Y_A$, and $(1 - Y_H)K_{S,H}$, $(4.57 - Y_A)/K_{S,A}$ (Spanjers and Vanrolleghem, 1995). This means that only for a given (or assumed) biomass concentration (X_H, X_A) and measured or assumed yield coefficient (Y_H, Y_A), it is possible to estimate $\mu_{max,H}$ and $\mu_{max,A}$, $K_{S,H}$, and $K_{S,A}$ separately.

The fractions of autotrophic biomass concentration X_A and X_H were not determined. Therefore, in what follows, the kinetics are related to the total biomass concentration. The measured Y_H, by addition of 20 mg COD HAc/l, was 0.78 g COD/g COD, the Y_A was assumed to be 0.24 g COD/g N. It must be stressed, however, that the reported parameter values are calculated using the above assumptions or measurements. Therefore, recalculation from the given parameter combination formulae allows to account for different yield values or biomass fractions than the ones used in our calculations. For instance, the real $\mu_{max,A}(\mu^*_{max,A})$ can be calculated using Equation (7) if the fraction (f_A) of X_A in the total biomass is known.

$$\mu^*_{max,A} = \frac{\mu_{max,A}}{f_A}. \tag{7}$$

The parameter estimation was performed under the assumption that the yield coefficients for the heterotrophic and autotrophic biomass were constant during the experiment. With the knowledge of the initial substrate concentrations (S_C and S_{NH_4} at t_0), the biokinetic parameters (μ_{max} and K_S) for both the C and N biodegradation processes can be obtained by fitting the $OUR_{ex}(t)$ as calculated with the Double Monod model (Equation 6) to the experimental $OUR_{ex}(t)$ data. The non-linear parameter estimation is based on the direction set algorithm of Brent (1973). The above estimation is done automatically with a software program called MOSIFIT (MOdel SImulator and FITter, available on request). An example of Double Monod kinetic parameter estimation is given in Fig. 3.

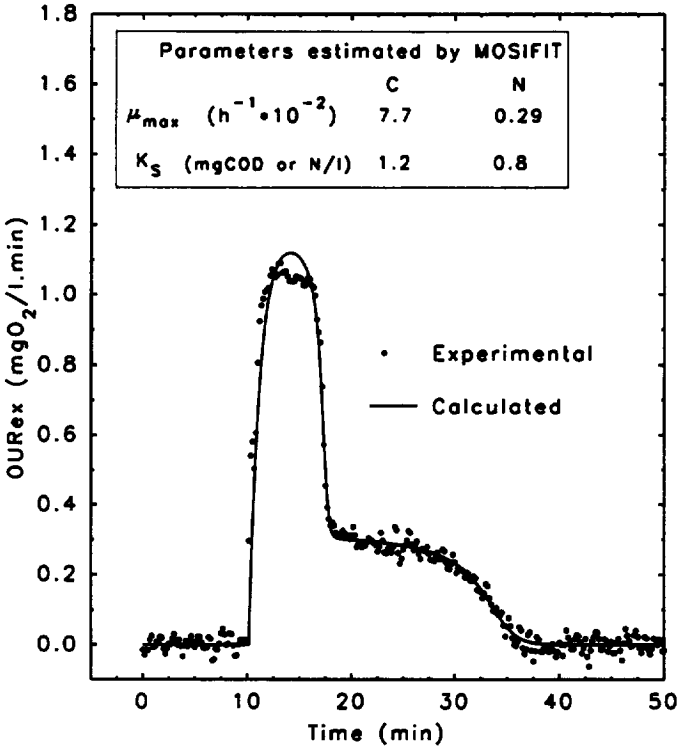

Fig. 3. An example of double Monod kinetics parameter estimation based on the OUR_{ex} profile (—, calculated OUR_{ex}; •, experimental OUR_{ex}).

Four batch experiments were run in order to check the reliability of the kinetic parameter estimation based on the OUR_{ex} profile resulting from the C–N-substrate mixture, as compared to that based on OUR_{ex} resulting of C and N added separately. In these experiments, pulses of 20 mg COD, 2.5 mg N and a mixture of 20 mg COD + 2.5 mg N/l were injected consecutively to the RODTOX reactor. The kinetic parameters for the single substrate (C or N) were estimated by the Single Monod model (Kong *et al.*, 1994) and those for the double substrate (C + N) were estimated by the Double Monod model. The results (Table 1) show no significant difference between the estimated parameters. It should be noted, however, that the coefficient of variation (CV) for the K_S is larger than that for μ_{max}.

Design of a suitable mixture of substrate

Proper design of the C–N-substrate mixture is essential for reliable parameter estimation (Vanrolleghem and Verstraete, 1993). Indeed the OUR_{ex}, due to the C- and N-substrate, must be well distinguished so that a reliable estimation of the two different aerobic process parameters based on the combined oxygen uptake rate ($OUR_{ex,TOT}$) is possible.

To find out a proper mixture of C + N substrate, it is necessary to know the minimum concentration required for the single substrates and the best C:N ratio. A first experiment was done to investigate the amount of a single substrate. The series of C-substrate concentrations applied was 5, 10, 20 and 30 mg COD/l and the series of N-substrate concentrations was 1, 2 and 2.5 mg/l. Carbon and nitrogen were alternatively dosed to avoid C stress towards nitrifying biomass. The resulting respirograms and corresponding OUR_{ex} profiles are shown in Fig. 4. The biokinetic parameters for C- and N-substrate degradation were estimated based on the OUR_{ex} predicted by the Single Monod model. The results (Fig. 4) show that the parameters are not dependent on the amount of substrate in the tested concentration range. The coefficient of variation of the estimated parameters is between 13% and 32%, which is satisfying. Therefore, 20 mg COD/l was chosen to find the suitable ratio between C and N.

Table 1. Comparison of kinetic parameter estimation based on OUR_{ex} curves of single substrate(C or N) and double substrate mixture (C + N) (n = 4)

	$\mu_{max,H}$ ($h^{-1} \times 10^{-2}$)		$K_{S,H}$(mgCOD/l)		$\mu_{max,A}$($h^{-1} \times 10^{-3}$)		$K_{S,A}$(mgN/l)	
	$\bar{x} \pm$ SD	CV%	$\bar{x} \pm$ SD	CV%	$\bar{x} \pm$ SD	CV%	$\bar{x} \pm$ Sd	CV%
C or N	5.38 ± 1.10	20.5	0.35 ± 0.14	40.0	2.50 ± 0.70	28.0	0.70 ± 0.46	65.7
C + N	4.58 ± 1.00	21.8	0.49 ± 0.16	32.7	2.70 ± 0.21	7.8	0.53 ± 0.17	32.1

	C -substrate (mg COD/l)				N-substrate (mgN/l)		
	5	10	20	30	1	2	2.5
μ_{max}($h^{-1}\times10^{-2}$)	7.74	9.81	9.95	7.97	0.44	0.43	0.34
K_S (mgCOD or N/l)	1.08	1.52	0.7	1.46	0.90	0.99	0.74
μ_{max} ($h^{-1}\times10^{-2}$)	8.87±1.17, CV = 13.2%				0.40±0.06, CV =15.0%		
K_S (mgCOD or N/l)	1.19±0.38, CV = 31.9%				0.88± 0.13, CV =14.8%		

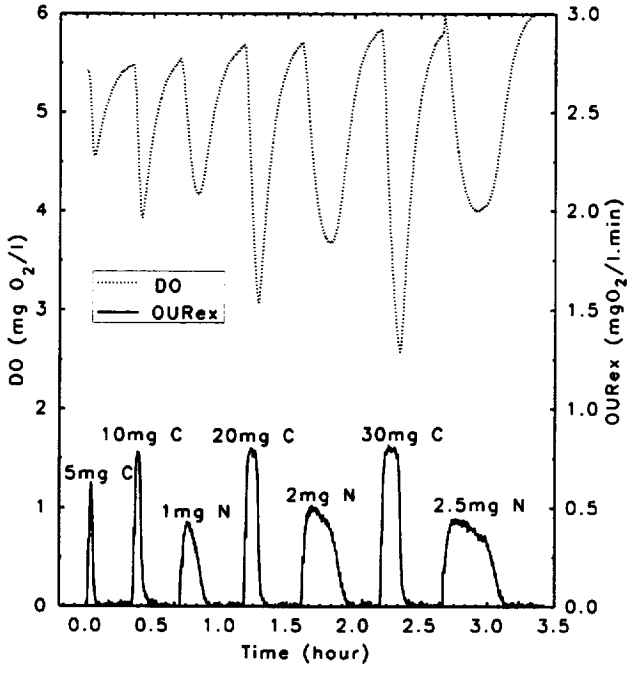

Fig. 4. Respirograms and corresponding OUR_{ex} profiles for different amounts of C and N substrates added to the RODTOX reactor.

The method used to find out the best C:N ratio for the mixture was based on the C and N substrate degradation time (which can be obtained from the OUR_{ex} curve). The time for complete N degradation is set twice as long as that for C degradation in order to obtain the combined oxygen uptake rate ($OUR_{ex,TOT}$) profile in which the OUR_{ex} due to the C- and N-substrate can be well distinguished. The 20 mg COD/l and 2 mg NH_4^+-N/l concentration pair fits this requirement. This C–N mixture was subsequently used for the RIKA tests with two toxicants (Cu^{2+} and CN^-). Figure 5 shows the resulting OUR_{ex} profiles for this substrate mixture with increasing toxicant (Cu^{2+} and CN^-) concentrations (see Table 2). It can be seen that, in case of the CN^- concentration series, the heterotrophic and autotrophic oxygen uptake rates can be well distinguished while this is not the case for Cu^{2+} concentration series (Fig. 5). To solve this problem a higher N (2.5 mg NH_4^+-N/l) concentration was used. The time to degrade such an amount of N is about 3 times longer than that for 20 mg COD/l. The resulting OUR_{ex} profile was satisfying for the three compounds tested (see Fig. 6).

ARIKA procedure

The whole ARIKA procedure consists of choosing a proper toxicant concentration range and performing batch experiments. The IC_{50} must be known approximately for the tested toxicant. The proper toxicant concentration series can be chosen by selecting four concentrations below the IC_{50} (decreasing by a factor of 2) and two higher concentrations (by a factor of 2).

The following batch experiment was performed. First, 20 mg COD/l as HAc is added for Kla estimation. Then the C–N-substrate mixture (20 mg COD + 2.5 mg NH_4^+-N/l) is injected. Subsequently, a series of mixtures (usually 5–7) of the mixed substrate with increasing toxicant concentrations is added. The sludge is not replaced between consecutive additions and the calculated cumulative toxicant concentration in the reactor is used for interpretation. Model-based interpretation establishes the dependence of the kinetic parameters on toxicant concentrations. This provides a data set on μ_{max} and K_S as function of toxicant concentration which allows the quantification of the effects of the

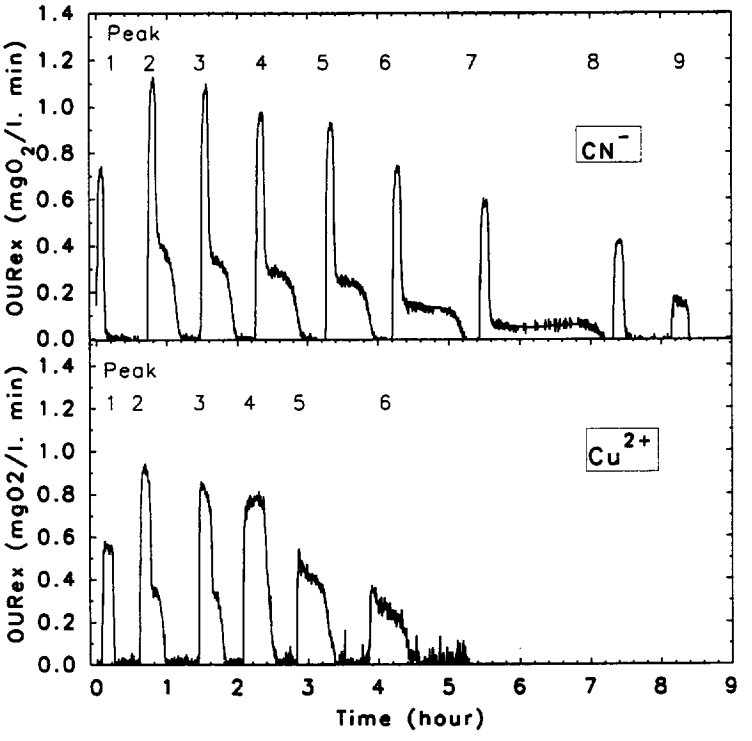

Fig. 5. OUR_{ex} profiles with C–N (20 + 2 mg/l) substrate with Cu^{2+} and CN^- as toxicants. The first peak is the one for pure acetate, the second one is the pure mixture of C–N, followed by a series of mixtures of the C–N substrate and toxicant (see Table 2).

inhibitor on the biogenic substrate removal. One such ARIKA test can be finished within 1 d.

Examples of applications

The test procedure was run for three compounds and was repeated three times for 3,5-DCP (OECD benchmark toxicant) to check for repeatability. The toxicant concentration series applied for the ARIKA test contained five to seven concentrations. The cumulative concentrations for the three chemicals tested are summarized in Table 2.

Typical respirograms and corresponding exogenous respiration rates for the three compounds are shown in Fig. 6. The impact of toxicants on the biodegradation of the C- and N-sources is clearly illustrated.

The kinetic parameters are calculated by the best fit of the Double Monod equation and summarized in Figs 7 and 8. The parameter estimation for each toxicant was performed until the toxicant concentration produces over 80% reduction of μ_{max}. The impact of the three toxicants on the C-oxidation is quite different. For CN^-, the μ_{max} decreases while K_S is essentially constant with increasing CN^- concentrations. For Cu^{2+}, μ_{max} also decreases with increasing toxicant concentrations. However, a different picture is observed for K_S. At Cu^{2+} concentrations below 10 ppm, K_S increases with Cu^{2+} concentrations. This is in agreement with previous findings (Kong *et al.*, 1994). Above this concentration K_S decreases. The phenomenon could be due to the fact that two groups of microorganisms respond in different ways to copper. As for 3,5-DCP, at low 3,5-DCP concentrations, μ_{max} increases with toxicant concentration. This can be explained by the fact that the toxicant acts as a respiration uncoupler (increase in oxygen consumption without generation of additional ATP). At higher 3,5-DCP concentrations, μ_{max} decreases while K_S increases with increasing toxicant concentration.

The impact of the three toxicants on nitrification is as follows: the μ_{max} for CN^- and 3,5-DCP decreases with increasing concentrations while K_S increases. For Cu^{2+}, the μ_{max} decreases and K_S is more or less constant with increasing toxicant concentrations.

Table 2. Cumulative toxicant concentrations for the ARIKA tests (ppm)

Conc. series	1	2	3	4	5	6	7
CN^-	0.013	0.038	0.088	0.188	0.388	0.788	1.588
Cu^{2+}	2.5	7.5	17.5	37.5	77.5	157.5	—
3,5-DCP	0.25	0.75	1.75	3.75	7.75	—	—

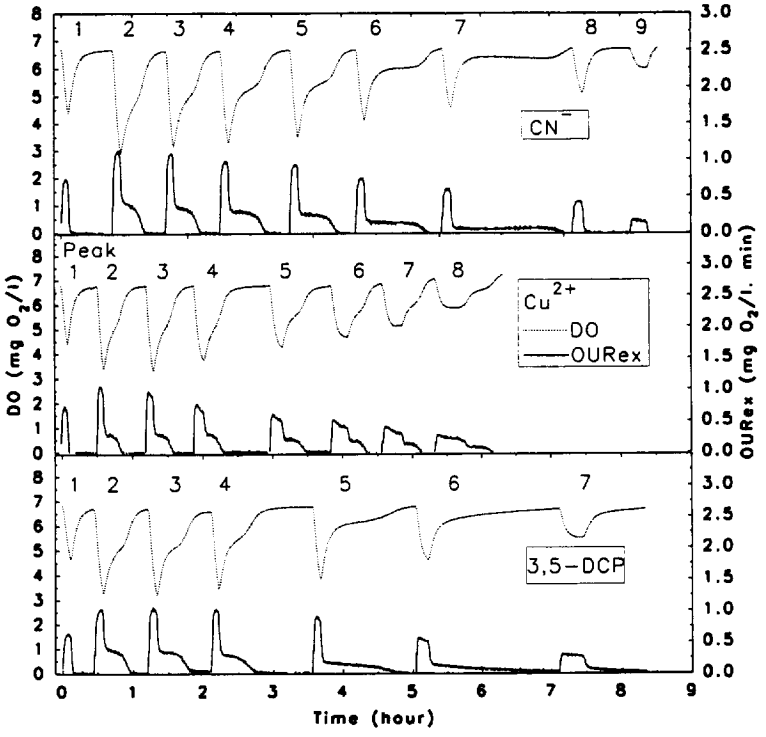

Fig. 6. Typical respirograms (DO, upper lines) and corresponding OUR_{ex} (lower lines) data obtained with three toxicants (CN^-, Cu^{2+} and 3,5-DCP, respectively). The first peak is the one for pure acetate, the second one is the pure mixture of C + N substrate, followed by a series of mixtures of the C–N substrate and toxicant (see Table 2).

For all tested compounds except Cu^{2+}, $\mu_{max,A}$ decreases faster than $\mu_{max,H}$, indicating that the nitrification process is more sensitive to CN^- and 3,5-DCP than the C-oxidation. This phenomenon is not observed for Cu^{2+}, suggesting that nitrification is more resistant to Cu^{2+}.

The repeatability of the test is illustrated by the 3,5-DCP data. The results, as indicated by the error bars in Fig. 8, show that the reproducibility is satisfactory considering that the experiments were performed on different days with different batches taken from one large sample of collected sludge which was stored at 4°C for less than 2 weeks.

DISCUSSION

Parameter estimation

The concentration of suspended solids (SS) or of volatile suspended solids (VSS) has been generally used as an indicator of the heterotrophic or autotrophic biomass concentration (Sharma and Ahlert, 1977; Verstraete and Van Vaerenbergh, 1986; APHA, 1989) in pure cultures or in activated sludge systems. In this study VSS was therefore used as an indication of the biomass concentration. The Monod constants reported in the literature (Table 3) vary within a wide range. Strong temperature and pH dependence of these parameters account for the variation (Sharma and Ahlert, 1977). The kinetic parameters estimated with MOSIFIT in the present study are in the range of values reported except $\mu_{max,A}$. The latter is due to the fact that nitrification biomass was not distinguished from the total biomass.

The fraction of nitrifiers ($f_{Nitrifier}$) in the sludge can be estimated from the relationship (Eckenfelder *et al.*, 1985)

$$f_{Nitrifier} = \frac{Y_{nitrifier} N_r}{Y_{nitrifier} N_r + Y_{heterotroph} BOD_r}$$

where

$Y_{nitrifier}$ = nitrifier yield coefficient (gCODbiomass/gN oxidized)
N_r = nitrogen oxidized (mgN/l)
BOD_r = BOD removed (mgO_2/l)
$Y_{heterotroph}$ = heterotroph yield coefficient (gCOD-biomass/gBOD removed)

Normal values for $Y_{nitrifier}$ and $Y_{heterotroph}$ are 0.24 and 0.67 (Henze *et al.*, 1987). The annual average BOD removed and nitrogen oxidized in the hospital wastewater treatment plant where the experimental sludge was produced were 333 and 54 mg/l (Kong, 1994), respectively. The estimated fraction of the nitrifiers in the sludge used is therefore 0.055. Taking this into account and using Equation (7), the value for $\mu_{max,A}$ becomes 0.045–0.08 h^{-1} and indeed falls within the reported range (Table 3). Similarly, for the heterotrophs one will find higher $\mu_{max,H}$ values if the heterotrophic fraction is taken into account.

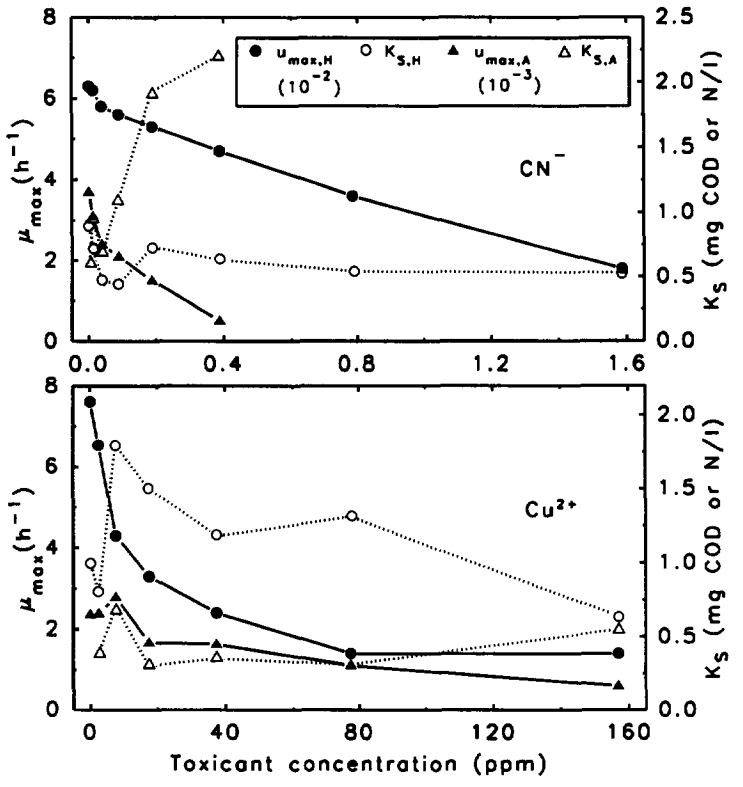

Fig. 7. Monod kinetic parameters for the biodegradation of the C- and N-sources in the presence and absence of Cu^{2+} and CN^- (●, $\mu_{max,H}$; ○, $K_{S,H}$; ▲, $\mu_{max,A}$; Δ, $K_{S,A}$).

Choosing a proper ratio of C and N is very important for the ARIKA test proposed. Normally this ratio can be chosen according to the differences in degradation times (the N degradation time should be approx. 2 times longer than the C degradation time). The time of N degradation should be three times longer than that of C degradation when the nitrifiers are less sensitive to a toxicant than the C-oxidizers (i.e. Cu^{2+}).

Simultaneous determination of the inhibition kinetics of both carbon oxidation and nitrification can be carried out with the proposed ARIKA procedure. It is labor-saving and simple compared to the separate determinations.

It was found that, due to numerical inaccuracy, it is difficult to estimate the biokinetic parameters when nitrification is inhibited more than 80%. Indeed, at such low levels of ammonium oxidation the plateau of $OUR_{ex,N}$ diminishes (see Fig. 6 especially for 3,5-DCP) and the parameter estimation with MOSIFIT software requires a defined plateau of the $OUR_{ex,N}$ in a OUR_{ex} profile.

The high coefficient of variation for K_S estimation, compared to the μ_{max} was also noted by Grady *et al.* (1989). These authors compared the biodegradation kinetic parameters estimated on the basis of OUR_{ex}, COD, DOC and biomass growth data sets, and found that the coefficient of variation was less than 10% for μ_{max} and 26–60% for K_S.

It is possible to improve the precision of parameter estimation by optimal experimental design procedures. Vanrolleghem *et al.* (1995) demonstrated that the accuracy of the parameter estimation may be increased by a factor of 2 through an additional pulse of substrate given at an appropriate time in the course of a respirogram. Another experimental design issue is that the amount of C-substrate in the C–N mixture can be varied according to the type of the toxicant. A higher amount of C-substrate in the mixture can be used for all toxicants except for a toxicant to which the nitrification is more resistant. In the latter case, one should lower the carbon level in the mixture.

Sensitivity of carbon oxidation and nitrification to a toxicant

It is assumed that the nitrification process is more sensitive to toxicants than carbon oxidation (Blum and Speece, 1991). Our experiments show that this is true for CN^- and 3,5-DCP but not for Cu^{2+}. This may be explained in the following way.

First, the nitrifiers are probably only susceptible to free Cu^{2+} (Braam and Klapwijk, 1981). Heavy metals however can form complexes with or absorb to other organics that are present in the wastewater or in the sludge and therefore become less toxic.

Second, the heterotrophic bacteria use organic molecules as substrates. As a consequence, these molecules can pass through the cell walls of the

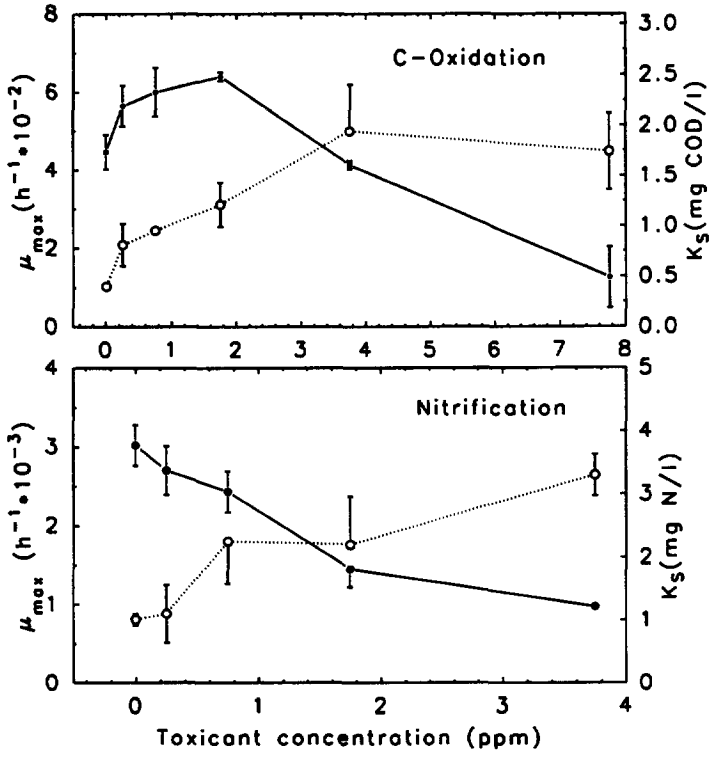

Fig. 8. Monod kinetic parameters for the biodegradation of the C- and N-sources in the presence and absence of 3,5-DCP, repeatability indicated by the error bar (●, μ_{max}; ○, K_S)

heterotrophic bacteria and are broken down intracellulary. Organic complexes formed with Cu^{2+} can also enter the cells and Cu^{2+} is set free when the organic complexes are degraded. The autotrophic bacteria do not have this capacity because they only use inorganic molecules as substrates (NH_4^+, CO_2, metal ions, etc). As a consequence, autotrophic (nitrifying) bacteria are exposed to less free Cu^{2+} than heterotrophic bacteria and are therefore less sensitive to Cu^{2+}. It is possible that this effect is more pronounced for Cu^{2+} than for other heavy metals because it is a well known fact that Cu^{2+} forms complexes more easily than other metals (Stumm and Morgan, 1981).

Inhibition types

Inhibitors (toxicants) are classified into four inhibition types (Table 4) according to the nature of their effect on μ_{max} and K_S. A similar approach has been taken for characterizing the effects of inhibitors on microbial activity in activated sludge (Hartmann and Laubenberger, 1968; Patterson and Brezonik, 1969; Volskay and Grady, 1988).

The results obtained in the present study indicate that CN^- is a noncompetitive inhibitor for C-oxidation and a mixed inhibitor for nitrification. Cu^{2+} is a mixed (at low concentration) and an uncompetitive inhibitor (at higher concentration) for C-oxidation, and a noncompetitive inhibitor for nitrification. 3,5-DCP is a mixed inhibitor for both C-oxidation and nitrification. The importance of the identification of the inhibition type is, as Volskay and Grady (1988) pointed out, that it determines the manner in which the substrate and inhibitor concentrations interact in regulating the substrate removal and biomass growth. It also governs the response of biomass to an inhibitory (toxic) shock

Table 3. Comparison of Monod parameters estimated with MOSIFIT with the literature data

	C-oxidation		Nitrification	
	μ_{max} ($h^{-1} \times 10^{-2}$)	K_S (mg COD/l)	μ_{max} ($h^{-1} \times 10^{-2}$)	K_S (mg N/l)
This study	4.6–8.9	0.4–1.2	0.25–0.40	0.53–0.88
Henze *et al.* (1987)[a]	25	20	3.33	1
Slide and Dare (1993)[b]	5.5–8.1	1.5–3		
Chudoba *et al.* (1989)[c]	1.7–11.4	0.4–3.2		
Drtil *et al.* (1993)[d]			3.15–4.2	0.28–0.61
Sharma and Ahlert (1977)[e]			1.9–9.1	0.06–5.6

Notes: (1) Measured with (a, b) wastewaster, (c) biodegradable organic compounds, (d) NH_4-N, μ_{max} calculated with $Y_A = 0.24$ and (e) not indicated. (2) Measurement temperature at 20°C (a) and not indicated (b–e).

Table 4. Definition of types of inhibition (Volskay and Grady, 1988)

Inhibitor type	Effect on μ_{max}	Effect on K_S
Competitive	None	Increase
Noncompetitive	Decrease	None
Uncompetitive	Decrease	Decrease
Mixed	Decrease	Increase

load (Santiago and Grady, 1990). For example, if an inhibitor acts in a competitive way, it will have no effect when the substrate concentration is high because the inhibitor does not have an effect on μ_{max}. A mixed inhibitor such as 3,5-DCP, on the other hand, is the worst type because it will have a negative effect regardless of the substrate concentration.

Control of the activated sludge wastewater treatment plant in case of intoxication requires that inhibitory phenomena are modeled. The information from RIKA tests is useful for this purpose. Linking the data produced by these tests with models as proposed by Santiago and Grady (1990) should give insights into the toxic effect and should allow the development of adequate control strategies for the treatment plant.

CONCLUSIONS

A fast and automated method is presented that allows the use of the RODTOX biosensor for off-line simultaneous assessment of inhibition kinetics for both carbon oxidation and nitrification. These results are achieved in the following way. First, a proper mixture of C–N biogenic substrates is chosen. Secondly, batch tests with different toxicant concentrations (usually 6–8 experiments) with the mixture of biogenic substrates are performed. Finally, the kinetic parameters of the biodegradation process of carbon oxidation and nitrification are estimated on the basis of the OUR_{ex} data sets obtained by running an automated data interpretation software. This allows to deduce the dependence of the kinetic parameters for both C and N degradation on the toxicant concentration.

Application of this procedure was demonstrated with three toxicants and repeatability was found to be satisfactory. The advantage of this method is that it is labor-saving and requires less experiments compared to existing methods. Additionally, the procedure and data interpretation can be performed automatically.

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