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An activated sludge-based biosensor for rapid IC_{50} estimation and on-line toxicity monitoring

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Abstract: The RODTOX (Rapid Oxygen Demand and TOXicity tester), an activated sludge-based respirographic biosensor, is a device for on-line monitoring of the short-term biochemical oxygen demand (stBOD) and potential toxicity of incoming wastewater on the basis of on-line interpretation of respirograms resulting from pulse additions of either calibration substrate or sample. The principle of toxicity detection is based on the comparison of calibration respirograms before and after receiving a potential toxicant. In this paper, the results of the RODTOX as an on-line toxicity monitor are presented. In addition, a simple and fast procedure to estimate the IC_{50} of a toxicant has been developed, and its validity and good repeatability demonstrated. The performance of this procedure is compared with that of the Microtox test.

Keywords: toxicity test, respiration inhibition, biosensor, IC_{50}.

1. INTRODUCTION

Nowadays, activated sludge processes are among the most widely used biological wastewater treatment systems in the world. The heterogeneous microbial community present in the sludge allows the system to be flexible, with regard to considerable fluctuations in the incoming wastewater composition. However, this capacity is limited. When the concentration of certain compounds increases, the inhibition (toxic) threshold concentration may be reached and this can adversely affect the organic and nutrient removal function. Therefore, it is important to screen and monitor on-line the potential toxicity of the influents to the microorganisms prior to their introduction into the biodegradation process.

In recent years, various microbial toxicity tests (Anderson *et al.*, 1988; Beaubien *et al.*, 1985; Bulich, 1986; King & Dutka, 1986; Koopman & Bitton, 1986; Larson & Schaeffer, 1982) have been described to determine toxicity towards activated sludge. In most of these tests, growth (agar inhibition zones, viability of cells, substrate uptake etc.), enzymatic activity (dehydrogenase), ATP contents, bacterial luminescence (e.g. Microtox), metabolic heat production (e.g. microcalorimetric techniques) and respiration rate have served as the indicating parameters of

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toxicity. Among these methods, the latter two allow rapid and continuous measurement of the overall biological activity of activated sludge.

The RODTOX (Rapid Oxygen Demand and TOXicity tester) biosensor, based on the on-line measurement of microbial respiration in an activated-sludge-filled reactor, allows rapid determination of waste content and potential toxicity of influents of a wastewater treatment plant (Vanrolleghem *et al.*, 1990). This paper will present the performance of the RODTOX as an on-line monitor of potential toxicity of wastewaters and discuss a fast and accurate procedure for estimating IC_{50} (concentration of a sample producing 50% respiration inhibition).

2. MATERIALS AND METHODS

2.1. Test chemicals

One organic compound (3,5-dichlorophenol, DCP) and two inorganic compounds (Cu^{2+} as $CuSO_4$; CN^- as KCN) have been used as test chemicals. All chemicals were of analytical grade.

2.2 RODTOX biosensor

The RODTOX biosensor, developed at the Laboratory of Microbial Ecology, University of Gent, in collaboration with Kelma NV, Belgium, consists of three main components (Fig. 1): a biological unit, the microprocessor with peripherals and software, and an electronic component that interfaces with the first two. The biological component consists of a reactor vessel, filled with 10 l of activated sludge, constantly aerated, stirred and thermostatted. Dissolved oxygen (DO) and pH electrodes are installed in the cover of the bioreactor. The respirometric data are analysed on-line by the microprocessor.

The principle of toxicity testing is based on a comparison of respirographic parameters, i.e. the peak slope (PS), peak height (PH) and peak area (PA), before and after the addition of possible toxicants (Fig. 2). Based on parameters of both respirograms, the percentage inhibition can be calculated.

The operating conditions of the RODTOX are given in Table 1. The calibration substrate was 10 g COD acetic acid (HAc) l^{-1} and the pulse dose was 150 mg COD HAc per injection.

The activated sludge was obtained from the aeration basin of the sewage treatment system of the Maria Middelares hospital, Gent. Since the hospital wastewater itself was only a minor part of this system's influent, the plant treated predominantly domestic sewage. The sludge was stored at 4°C.

2.3 Microtox

The principle of the Microtox is to measure the change in light output of a luminescent bacterium (*Photobacterium phosphoreum*) resulting from the addition of a toxicant. From a series of toxicant concentrations (usually four), the EC_{50} can be calculated; this is the effective concentration of a toxicant causing a 50% reduction in light emission when compared with the baseline level (Bulich, 1986). The test procedure is described in detail in the Beckman operating manual.

3. RESULTS

3.1 On-line toxicity detection

The on-line monitoring of toxicity is automated through a software routine by which a calibration substrate is injected at regular intervals, or whenever toxicity is suspected. The toxicity limit can be preset to activate a toxic alarm.

The sensitivity to detection of priority pollutants is presented in Fig. 3. Depending on the toxicant, different concentrations ranging from 1 to 5000 ppm resulted in significant sludge inhibition. It is very important, however, to mention that the results obtained are dependent on the source of the sludge. This is caused by adaptation of the sludge to the waste composition. Therefore, relevant results can be obtained only if the sludge used in the RODTOX is the same as the sludge in the plant to be controlled.

A practical example of the capability of the RODTOX to detect a phenol (1.2 g l^{-1}) shock loading is shown in Fig. 4. In this run, the RODTOX automatically performed a calibration cycle (CAL) every three sample measurement cycles. The peak slope (PS) of the calibration cycle before the phenol pulse started was 1.56 mg O_2 l^{-1} min^{-1}. It decreased to 0.22 mg O_2 l^{-1} min^{-1} in the calibration respirogram performed after receiving the phenol pulse. The

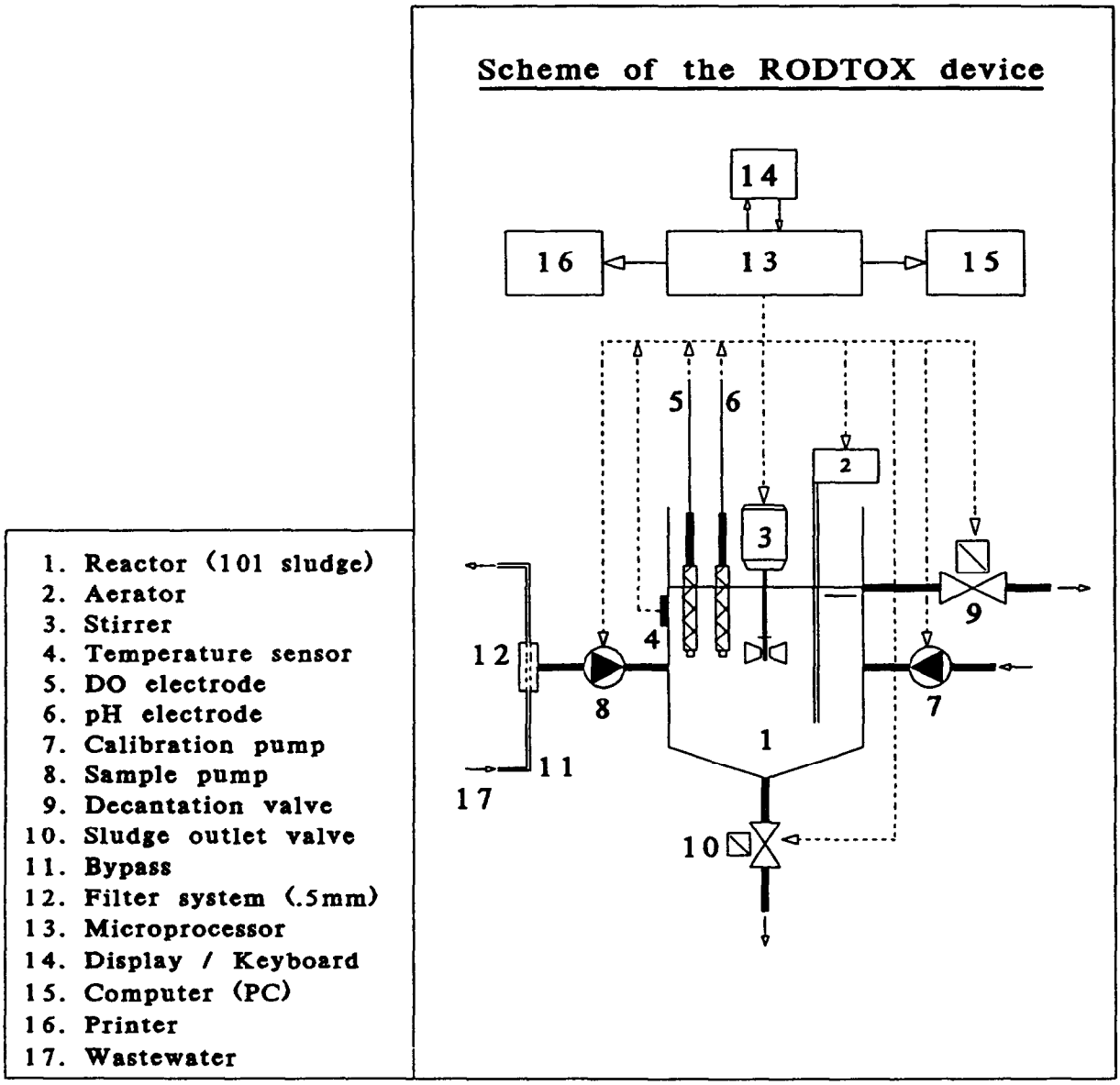

Fig. 1. Schematic of the RODTOX biosensor.

resulting output of the RODTOX was 86% PS inhibition. This caused an alarm because the value was above the preset alarm limit of 20%.

3.2 IC_{50} estimation

3.2.1 Principle of the cumulative exposure procedure
To estimate the IC_{50} of a toxicant with the RODTOX apparatus, one can use a traditional toxicity testing procedure. In this method, called the 'sludge replacement' procedure, the activated sludge is withdrawn after the toxicity of one toxicant concentration is measured, and the bioreactor is then filled with new sludge. However, this procedure is time consuming and laborious; a cumulative exposure procedure was therefore developed as an alternative.

In the proposed procedure, one calibration substrate and a series of mixtures of calibration substrate with increasing toxicant concentration (usually 4–5) are injected consecutively. The inhibition percentages of respirographic parameters are then calculated by comparing them with the first calibration substrate peak. The IC_{50} can be calculated from the inhibition

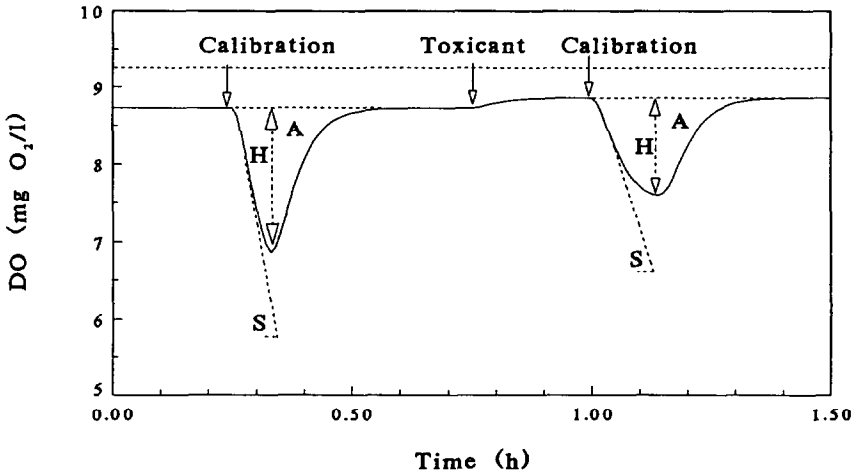

Fig. 2. Principle of the RODTOX toxicity test. S = peak slope (PS); H = peak height (PH); A = peak area (PA).

TABLE 1 RODTOX operating conditions

Parameter	Value
pH	7·0 ± 0·2
Mixed liquor (l)	10·0
Temperature (°C)	25·0
Sludge conc. (g SS l^{-1})	4·5 ± 0·5
Respira[a] (mg O_2 l^{-1} min^{-1})	0·23 ± 0·02
Baseline DO (mg O_2 l^{-1})	6·0 ± 0·5

[a]Endogenous respiration.

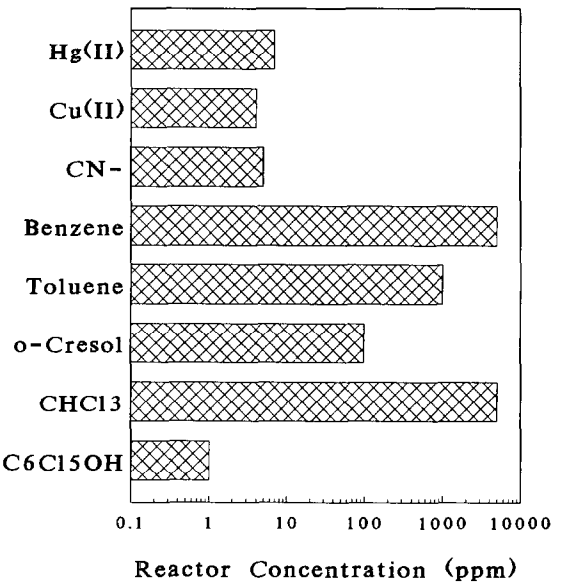

Fig. 3. Toxicity detection limits of some principal water pollutants.

percentages corresponding to this series of toxicant concentrations. The IC_{50} is defined here as the concentration of a toxicant causing 50% reduction of the respirographic parameter compared with that of the calibration peak.

Before a toxicity test run started, the endogenous respiration rate of the sludge was measured by a 'Respira' test as an indication of sludge activity. The Respira level was then used to control the quality of the test.

A detailed description of the procedure is shown in Fig. 5.

3.2.2 IC_{50} values estimated by both cumulative exposure and sludge replacement procedures

To avoid changes of the microbial community in the activated sludge, a particular batch of sludge was used for assessing IC_{50}s with both procedures. Typical respirographic curves obtained from the cumulative procedure for assessing IC_{50}s of 3,5-DCP, Cu^{2+} and CN^- are given in Fig. 6. The caption to this figure explains the successive peaks in each diagram. From the results, it can be seen that the slopes (PS) of the descending part of these respirograms become less steep with increasing toxicant concentration.

The inhibition percentages of PS were calculated by comparing the PS of the calibration peak (PS_{cal}) with the PS of a respirogram corresponding to the injection of a certain toxicant concentration (PS_{tox}), using the equation:

$$\% \text{ inhibition} = \frac{PS_{cal} - PS_{tox}}{PS_{cal}} \times 100$$

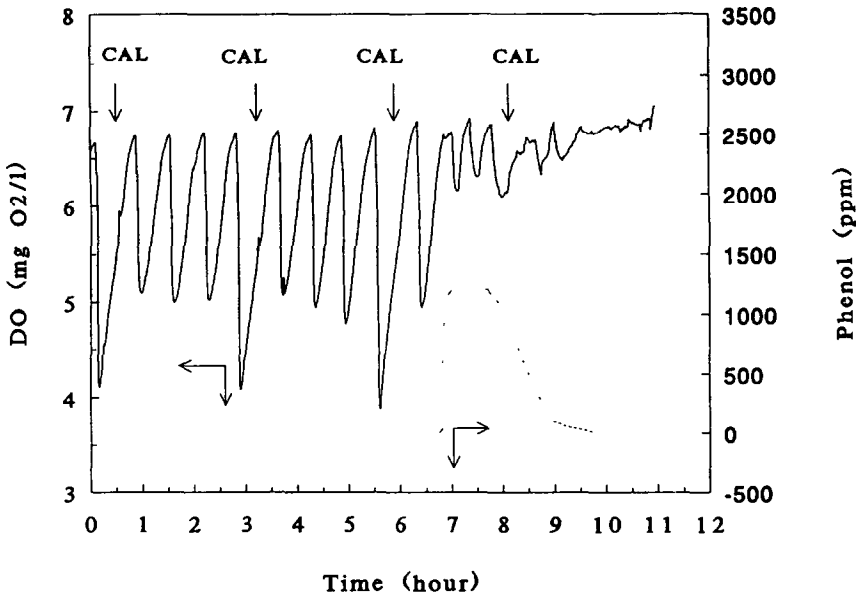

Fig. 4. Respirograms of the RODTOX monitoring a pulse of 1·2 g phenol l^{-1}. The solid line represents DO concentration and the dotted line phenol concentration.

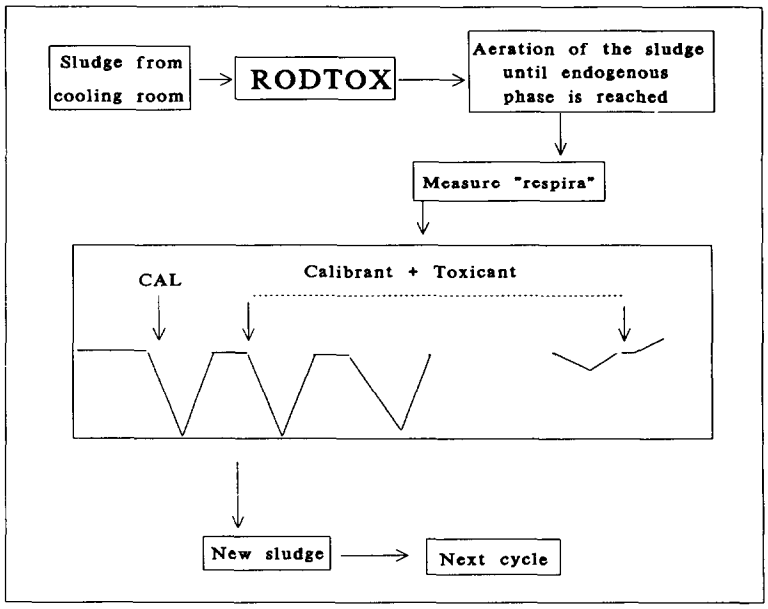

Fig. 5. Cumulative toxicity testing procedure. First, a 'Respira' test is performed to assess the endogenous respiration, then a calibration substrate injection follows. The following peaks relate to consecutive additions of a mixture of calibration substrate with increasing toxicant concentration.

For both procedures, the concentration-response curves of 3,5-DCP, Cu^{2+} and CN^- with respect to PS inhibition are shown in Fig. 7. The inhibition percentages of PS show a linear relationship with the log concentration for both procedures, and the results are highly repeatable.

The IC_{50} (PS) estimation of copper and cyanide showed no significant difference between the two procedures (Table 2). The IC_{50} (PS) of 3,5-DCP estimated by the traditional procedure, however, is nearly twice as high, suggesting the new procedure has a higher

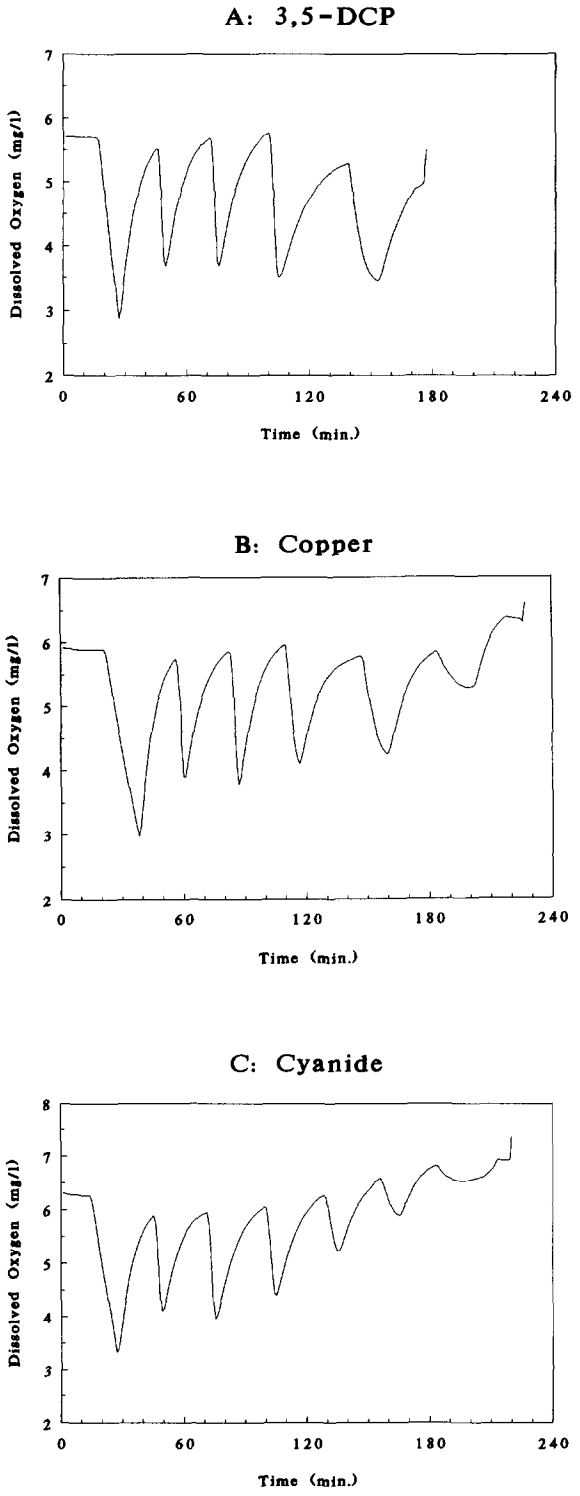

Fig. 6. Typical respirographic curves obtained from the cumulative procedure for assessing IC_{50}s of 3,5-DCP (A), Cu^{2+} (B) and CN^- (C). The first peak is a Respira test (to assess the endogenous respiration), the second is calibration and the following are consecutive additions of a mixture of calibration substrate and toxicant.

sensitivity. Furthermore, an IC_{50} estimation with the cumulative procedure takes less time and does not require a change of sludge.

3.2.3 Validity of RODTOX method for toxicity testing

The validity of the RODTOX method for toxicity testing was checked by estimating the IC_{50} value of the OECD reference toxicant 3,5-DCP (OECD, 1987). It was estimated with four batches of sludge collected at different times; the results are shown in Table 3. These values are well within the OECD accepted range (5–30 ppm). IC_{50} (PH) and IC_{50} (PA) are almost twice as much as IC_{50} (PS), indicating that the maximum substrate oxidation rate (indicated by PS) is the most sensitive parameter. The variation coefficients of IC_{50} estimated by PS, PH and PA are less than 22%. This indicates that the repeatability of the method is quite satisfactory.

3.2.4. Repeatability and sensitivity comparison with the Microtox test

Comparison of the variation coefficients of the IC_{50}s estimated by the RODTOX and Microtox methods shows that the repeatability of the RODTOX method is as good as or even better than that of the Microtox method (Table 4). The sensitivity of both methods, however, depends on the toxicant. The Microtox test is more sensitive than the RODTOX method for 3,5-DCP and copper, while the opposite holds for cyanide.

4. DISCUSSION

Occasional spills at an industrial site may cause unusually high concentrations of certain chemicals which have adverse effects on the performance of the biological wastewater treatment system. An accidental spill should be detected early enough to alert treatment plant operators to take necessary remedial or control measures. In such a case, a holding basin with an automatic bypass can be activated by the RODTOX biosensor's alarm. The toxic wastewater in the holding basin can then be pretreated, diluted or released gradually to the system.

The advantages of the apparatus are the easy replacement of the biomass in the bioreactor so that it is suitable for *in situ* continuous toxicity monitoring,. Moreover, as the RODTOX uses the same community of microorganisms as in

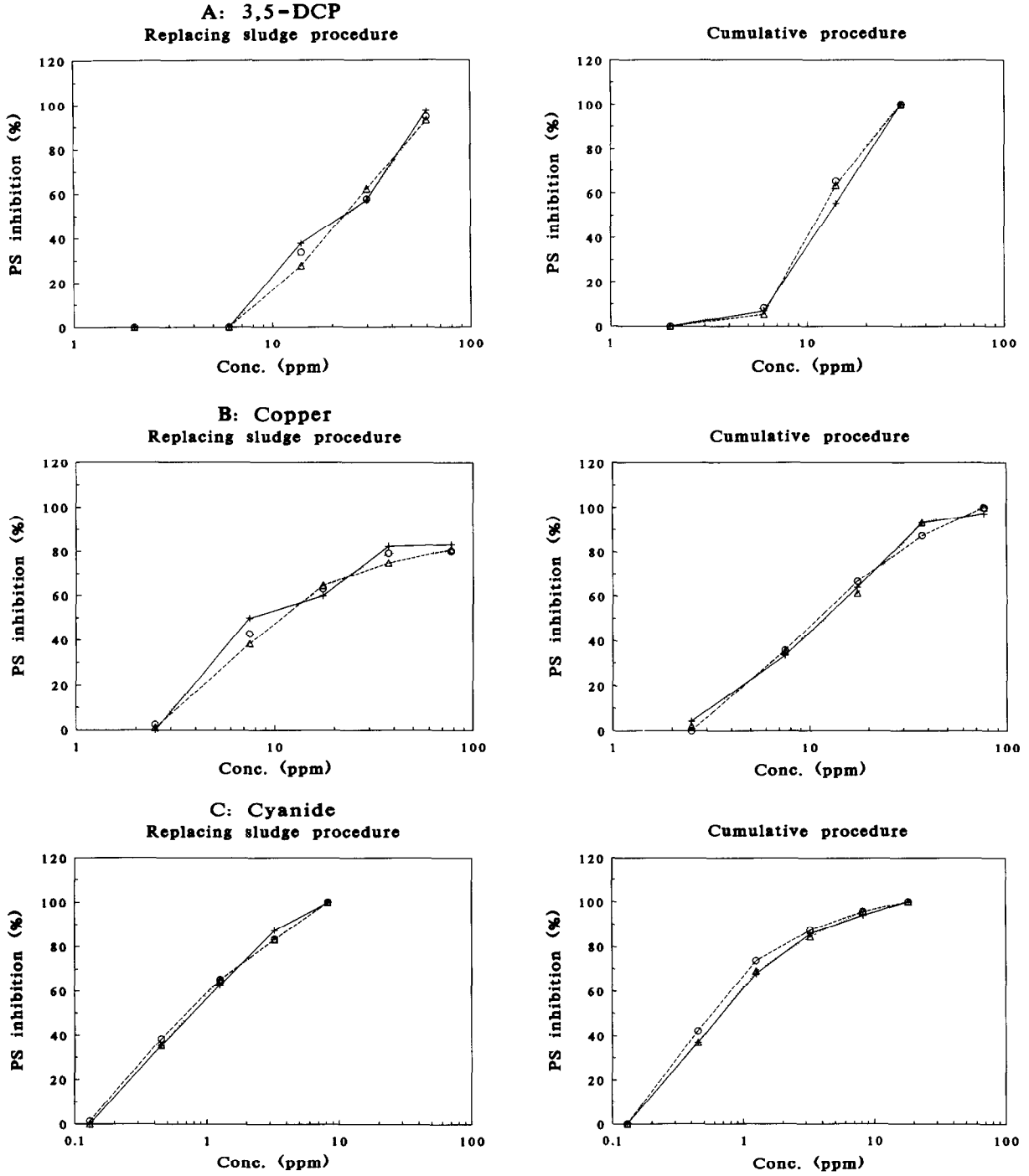

Fig. 7. Concentration-response curves of 3,5-DCP (A), Cu^{2+} (B) and CN^- (C) for both the sludge replacement procedure (left) and the proposed cumulative procedure (right). For each concentration, three replicates are shown.

activated sludge plants, it is ecologically relevant to them.

IC_{50} estimation is important because acute toxicity testing is the first step in determining the acceptable levels of a given toxicant in municipal and industrial discharge as well as in the aquatic environment (Shieh & Yee, 1985). The IC_{50} values estimated with both procedures showed the validity of the RODTOX biosensor when used as an off-line toxicity tester. However, estimating

TABLE 2 IC_{50} (PS) (ppm) estimation with both procedures for three toxicants

	Cumulative procedure	Sludge replacement procedure
3,5-DCP	11·7	21·7
Cu^{2+}	11·4	12·8
CN^-	0·78	0·81
Test period (h)	5	12
Amount of sludge (l)	10	40

TABLE 3 Repeatability of IC_{50} (ppm) values of 3,5-DCP for different batches of sludge, estimated with the cumulative procedure

Date	IC_{50}(PS)	IC_{50}(PH)	IC_{50}(PA)
18/12/90	9·7	12·7	20·2
12/3/91	13·5	21·6	21·6
22/3/91	11·7	20·0	20·0
03/4/91	12·5	20·5	20·5
Av. ± SD	11·9 ± 1·6	18·7 ± 4·1	20·6 ± 0·7
CV (%)	13·5	21·6	3·4

IC_{50} with the proposed cumulative exposure procedure showed advantages such as time- and labour-saving when compared with the sludge replacement procedure. Moreover, the cumulative procedure seemed more sensitive. The results showed that the maximum substrate oxidation rate of activated sludge (indicated by peak slope) is the parameter most sensitive to inhibitory effects. This is probably due to the fact that the maximum respiration rate is directly related to active biomass in the activated sludge. Toxicity may not be reflected by peak area (PA) because this parameter actually reflects the amount of substrate oxidized (Vanrolleghem *et al.*, 1990). Although some biomass may be affected by the toxicant, the viable part of the activated sludge is still able to oxidize the substrate completely (resulting in an identical PA), provided a longer degradation time is given.

In general, a lower concentration of bacteria used in a test results in a higher sensitivity to the toxicant (King & Dutka, 1986). This can be seen by comparing the IC_{50} results of 3,5-DCP and copper obtained by the BOD inhibition test, ISO methods A and B, OECD and RODTOX methods (Table 5). The effect is probably a result of the varying ratio of inhibitor to microorganisms. It can be seen from Table 5 that the BOD method with the lowest number of organisms, in general, tends to be the most sensitive, giving the lowest IC_{50} values.

However, this general rule is not applicable for toxicity results of the OECD and RODTOX methods. The IC_{50} of 3,5-DCP estimated by the RODTOX procedure is lower than that of the OECD method, and the IC_{50} of copper estimated by the RODTOX is almost three times lower than that obtained by the OECD method. On the other hand, the concentration of microorganisms in the RODTOX procedure is three times higher than in the OECD method. This comparison suggests that the RODTOX method is more sensitive to 3,5-DCP and copper than the standard OECD method.

The Microtox test is widely used for toxicity testing (Bulich, 1986) since it is rapid, simple and inexpensive to perform, and allows EC_{50} estimation with good repeatability (CV = 5–31%). The repeatability of the RODTOX method was, in the trial described, as good as or even better than that of the Microtox. The different sensitivity of the Microtox and the RODTOX tests can be explained by the characteristics of each test, e.g. the contact times are different. Two more factors

TABLE 4 Comparison of repeatability and sensitivity between Microtox and RODTOX methods

Toxicant	3,5-DCP		Cu^{2+}		CN^-	
	RODTOX	Microtox[a]	RODTOX	Microtox	RODTOX	Microtox
I(E)C_{50} (ppm)	11·70	1·81	12·80	0·31	0·81	3·89
Sd	0·75	0·08	1·20	0·04	0·01	0·74
CV (%)	6·4	4·4	9·4	12·9	1·2	19·0

[a]Contact time: 5 min for 3,5-DCP and CN^-, 15 min for Cu^{2+}

TABLE 5 Comparison of sensitivity of the RODTOX method with other methods in assessing toxicity of chemicals towards activated sludge (IC_{50} values)

Method	3,5-DCP	Cu^{2+}	Cells ml^{-1}	Reference
BOD inhibition	7(3–19)	4·5	10^3	King & Dutka (1986)
ISO method A	10(4–25)		10^6	King & Dutka (1986)
ISO method B	6(5–16)	123	10^{7-8}	King & Dutka (1986)
	22	17	10^{7-8}	Dutka *et al.* (1983)
OECD method	20·0		10^8	Yoshioka *et al.* (1986)
		29	10^8	King & Dutka (1986)
	12·2		10^8	Klecka & Landi (1985)
RODTOX method[a]	11·7	11·4	3×10^8	This study

[a] IC_{50} (PS).

can be mentioned. First, the quantity of microorganisms used in the RODTOX method is much higher (~ 300 times) and secondly, activated sludge is known to adsorb toxicants, resulting in a decrease of their effective concentration in the test medium, i.e. affecting the bioavailability of the test chemicals.

Compared with the RODTOX method, a lower sensitivity of the Microtox method to cyanide was observed. The poor assessment of toxicity for effluents containing cyanide was also noticed by Qureshi *et al.* (1982). More recently, Reteuna *et al.* (1989) pointed out that the Microtox test does not seem to be well suited for predicting the possible toxic effects of wastewaters on the activated sludge treatment plant. It was stated that the Microtox method uses a pure culture of marine species and, therefore, could not necessarily be expected to behave in the same way as a community of freshwater species (King and Dutka, 1986). This comment may, however, be applied to all toxicity assessments using single species.

5. CONCLUSIONS

The RODTOX biosensor was shown to be capable of detecting the potential toxicity of incoming wastewater to the biotreatment processes on-line.

A procedure which allows the use of the RODTOX for the fast estimation of the IC_{50} (50% inhibition concentration) of chemicals has been developed. It is easy, valid, cost effective and repeatable. It compares well with the Microtox test and, moreover, is directly relevant to the plant to be controlled.

The maximum oxidation rate of the substrate was shown to be the most sensitive indicator of toxicity towards activated sludge. The cumulative exposure procedure seems more sensitive than the traditional sludge replacement procedure.

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