Application of a *Tetrahymena pyriformis* bioassay system for the rapid detection of toxic substances in wastewaters

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Abstract

A bioassay technique based upon the respiratory response of *Tetrahymena pyriformis* to intoxication, which gives results within 10 min, was used to evaluate the quality of a number of effluents from three wastewater treatment plants in the Pretoria-Johannesburg area. Toxic activity was detected in both treated and untreated wastewater effluent. The technique was shown to be sufficiently sensitive to monitor toxicity in raw and treated wastewater effluent, and to evaluate the efficiency of the various treatment stages to remove toxicants from raw waters.

Introduction

Successful control of water quality necessitates efficient monitoring procedures. Modern sophisticated physical and chemical analytical techniques provide valuable information on the presence of toxic substances in wastewaters (Leh and Chan, 1974; Hattingh, 1979), and have become the primary means whereby toxic discharges are identified. It is not always possible, however, to predict adverse effects of wastewater discharges on the biota of receiving aquatic ecosystems by physical and chemical analyses alone (Kingsbury and Rees, 1978; Cairns and Gruber, 1979). Tests involving the use of biological organisms are, therefore, being increasingly used to supplement physical and chemical determinations. Over the past decade the number, variety and complexity of biological test procedures have steadily increased and no longer simply involve assessments of the acute lethal effect of complex wastes upon aquatic life (Cairns and Van Der Schalie, 1980; Maciorowski, Sims, Little and Gerrard, 1981). Long-term effects of intoxication upon physiological functions such as growth, reproduction, metabolism and behaviour have provided information whereby safe levels of wastewater discharges can be established (Hunter, 1978). Such tests are, however, tedious and time consuming. Considerable attention has therefore been given to the development of techniques which will rapidly detect toxicants with the same sensitivity as long-term tests (Zullei and Benecke, 1978; Bulich and Greene, 1979; Williamson and Johnson, 1981).

A rapid bioassay technique, based upon the effect of intoxication on oxygen uptake rate of the protozoan *Tetrahymena pyriformis*, has recently been described (Slabbert and Morgan, 1982). The technique was successful in detecting chronic levels of individual toxicants within 10 min. This paper investigates the viability of the technique to detect harmful levels of pollution in wastewaters from three separate wastewater treatment plants in the Pretoria-Johannesburg area. The use of the technique as a method of monitoring the quality of potentially toxic wastewater discharges is assessed.

Materials and Methods

Preparation of the test organism

T. pyriformis, strain W (originally obtained from the Culture Centre of Algae and Protozoa, Cambridge, UK), was grown axenically at 27 °C in a 10 g/ℓ proteose peptone medium. Logarithmic growth phase cells were used throughout the study. An adequate cell concentration was obtained every 24 h using a 2% inoculum (v/v) for subculturing. Cells used for toxicant evaluation were suspended in Osterhout salt solution (Taylor and Strickland, 1935) according to the filtration method described by Slabbert and Morgan (1982). The cell suspension was diluted to a concentration which yielded an oxygen uptake rate of approximately 8% per min. Cell suspensions were maintained at 27 °C for 30 min before being used in the assays. Approximately 50 mℓ of a cell suspension was prepared for each test series, and employed within 2 h of preparation to avoid any interaction between the products of cell metabolism and toxicants.

Oxygen monitoring apparatus

A biological oxygen monitoring system (YSI model 53, Yellow Springs Instrument Co., Yellow Springs, Ohio, USA), consisting of an electronic unit, an oxygen probe and a standard bath unit equipped with airtight test chambers and a magnetic stirrer, was employed to examine the respiration rate of *T. pyriformis*. A potentiometric recorder (Linear model 355, Linear Instruments Corp., Irvine, California, USA) was linked to the system to provide a permanent graphic record of oxygen uptake rate, the chart speed being regulated to 1 200 mm/h. The temperature within the test chambers was stabilized at 27 °C using a constant temperature water circulator (Lauda K2R electronic, Messgeräte-Werk Laude/Tauber, FDR). Calibration of the apparatus was carried out with air-saturated deionized water.

Test samples

Samples were collected from three different wastewater treatment plants in the Pretoria-Johannesburg area. Plant A receives separated industrial and domestic effluents, plant B a mixture of both industrial and domestic wastewater, and plant C primarily domestic wastewater. Twenty-four hour composite samples of both raw and treated effluents (Table 1) were collected on three separate occasions between October 1980 and February 1981. Samples were decontaminated using a 0,45 μm Sartorius membrane filter and maintained at 10 °C until evaluation.

T. pyriformis feeds on bacteria and readily ingests particulate materials (Hill, 1972). Since the test organism was suspended in a salt solution without nutritious matter, the addi-

TABLE 1
WASTEWATER EFFLUENT SAMPLES

Sample No.	Type
A1	Plant A: Raw industrial wastewater
A2	Plant A: Raw domestic wastewater
A3	Plant A: Humus tank effluent
A4	Plant A: Activated sludge effluent
B1	Plant B: Settled wastewater
B2	Plant B: Biofilter effluent
B3	Plant B: Humus tank effluent
C1	Plant C: Raw wastewater
C2	Plant C: Humus tank effluent
C3	Plant C: Denitrification plant effluent

tion of these materials might cause an increase in respiration rate. Preliminary tests carried out on the unfiltered water samples showed that the raw wastewater samples (containing many bacteria and particles) produced a greater increase in oxygen uptake rate than the filtered samples. In order to examine toxic effects it was thus necessary to decontaminate the test samples.

Bioevaluation

Tests were performed according to the procedure outlined by Slabbert and Morgan (1982). For each test, 4,75 mℓ of the prepared cell suspension were aerated for 5 min in a test chamber. Oxygen monitoring commenced immediately after the probe had been inserted into the test chamber. The effect of test samples on respiration rate was examined by injecting 0,25 mℓ of the test solution directly into the chamber containing the cell suspension. Test samples were introduced when dissolved oxygen within the chamber had decreased to approximately 63%. Oxygen uptake was recorded continuously before, during and after injection of the test sample.

A convenient reference oxygen uptake rate, before sample introduction, was 8% per min, recorded as a straight line having a 45 ° slope (Figure 1, line a). The mixing of test sample with the cell suspension is represented on the graphical output as a line parallel to the time axis (Figure 1, line b). Typical effects of test solutions are shown in Figure 1, lines c to f. Deionized water causes a change in oxygen uptake rate due to a dilution of the cell suspension (Figure 1, lines c), and this line can be used as a convenient control for test graphs. Lines d and e illustrate, respectively, an increase and a decrease in oxygen uptake rate. An accelerating decrease in oxygen uptake rate is represented by line f in Figure 1, and usually indicates progressive lethality in the cell suspension.

The effect of test samples was established by comparing the oxygen uptake rate after sample injection (test) with the reference uptake rate. (Using continuous recording, the determination of cell populations and actual oxygen concentration was not necessary.) Variation in reference uptake rates was eliminated by calculating all results in relation to a standard reference uptake rate of 8% per min according to the formula:

$$\frac{\text{test oxygen uptake rate}}{\text{reference oxygen uptake rate}} \times 8\% \text{ per min.}$$

Oxygen uptake rates were determined at 1, 2, 3, 4 and 5 min after mixing of test sample and cell suspension. Each test was repeated six times and a control test (deionized water) performed after every four to six tests. Student's t-test was applied to

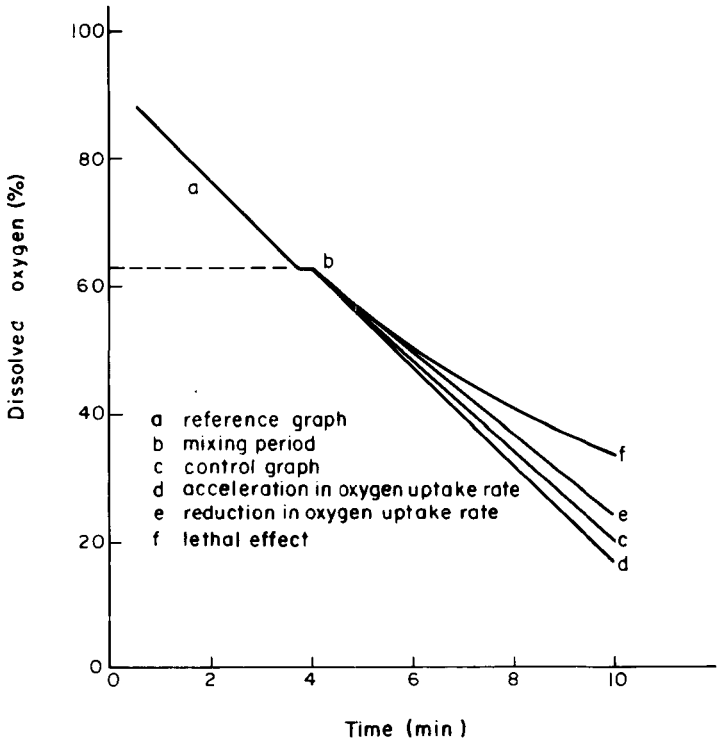

Figure 1
Typical effects of test solutions on oxygen uptake rate of Tetrahymena pyriformis

establish whether average test results differed significantly from average control results at the P = 0,05 level.

Toxicity tests with individual toxicants showed that sublethal concentrations may cause a decrease or an increase in the oxygen uptake rate of *T. pyriformis* (Slabbert and Morgan, 1982). Toxicants such as ammonia, cyanide, mercury and phenol decreased respiration rate while cadmium, copper, lead, parathion, pentachlorophenol and zinc produced an increase. Both an increase and a decrease in oxygen uptake rate can thus be used as an indication of toxicity if water samples were to be tested. The larger the change in uptake rate, the higher the toxicity level (Slabbert and Morgan, 1982).

Waters may contain chemical substances, e.g. ferrous iron, sulphites and sulphides, which in themselves have an oxygen demand. In order to ensure that an increased oxygen uptake rate (Figure 1, line d) was due to toxicity and not because of the presence of oxygen reducing chemicals in the wastewaters preliminary tests were carried out injecting test samples into aerated Osterhout salt solution without the test organism. Within the first 20 to 30 s after sample addition, the same period of time it takes for mixing of test samples and cell suspensions (Figure 1, line b), a change of 1 to 2% in saturation occurred (because of different concentrations of dissolved oxygen in the water samples). A constant record of % dissolved oxygen thereafter indicated the absence of oxygen reducing chemicals.

As the total salt concentration of complex water samples may cause responses from organisms when no specific toxicant is present the effect of various concentrations of Osterhout salt solution (Table 2) and of reconstituted hard waters (Table 3) on oxygen uptake rate was examined. The salt concentrations of the wastewater samples (Tables 5, 6 and 7) were below those of the twentyfold concentrates of Osterhout salt solution, while hardness values corresponded with that of a reconstituted fresh water in the 'hard' range, i.e. 160 – 180 mg/ℓ $CaCO_3$ (United States Environmental Protection Agency, 1975). In addition the effect of pH on respiration rate was established using deionized water adjusted to various pH levels (Table 4).

TABLE 2
EFFECT OF OSTERHOUT SALT SOLUTION ON OXYGEN UPTAKE RATE OF *TETRAHYMENA PYRIFORMIS*

Concentrates of Osterhout solution	Oxygen uptake rate at time indicated (min)				
	1	2	3	4	5
Control	7,2	7,2	7,2	7,2	7,2
20-fold	7,2	7,2	7,2	7,2	7,2
100-fold	7,3	7,3	7,3	7,3	7,3
150-fold	7,3	7,3	7,3	7,3	7,3
200-fold	7,3	7,3	7,3	7,3	7,3
400-fold	7,4	7,2	7,0	7,0	7,0
1000-fold	7,7	7,7	7,5	7,1	6,6

Results are expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions.

TABLE 3
EFFECT OF WATER HARDNESS ON OXYGEN UPTAKE RATE OF *T. PYRIFORMIS*

Hardness (mg/ℓ $CaCO_3$)	Oxygen uptake rate at time indicated (min)				
	1	2	3	4	5
Control	7,2	7,2	7,2	7,2	7,2
10 – 15 (very soft)	7,2	7,2	7,2	7,2	7,2
160 – 180 (hard)	7,2	7,2	7,2	7,2	7,2
280 – 320 (very hard)	7,3	7,3	7,3	7,3	7,3

Results expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions.

TABLE 4
EFFECT OF pH ON OXYGEN UPTAKE RATE OF *T. PYRIFORMIS*

pH	Oxygen uptake rate at time indicated (min)				
	1	2	3	4	5
Control (approx 5,5)	7,2	7,2	7,2	7,2	7,2
1	2,8	1,7	0,8	0,2	0
2	4,6	3,8	3,2	2,9	2,6
2,5	6,4	6,0	5,7	5,6	5,6
3	7,5	7,5	7,5	7,5	7,5
4	7,2	7,2	7,2	7,2	7,2
8	7,2	7,2	7,2	7,2	7,2
9	7,3	7,3	7,3	7,3	7,3

Results expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions.

Results and Discussion

All results are expressed in relation to a reference oxygen uptake rate of 8% per min and represent the mean values of six determinations. The coefficient of variation for control (deionized water) and test results generally varied from 0 to 2%. In cases of large increases in respiration rate (samples A1 and A2) variation was slightly greater (coefficient of variation: up to 4%), and in cases of lethality (sample A1 and pH values $\leq$ 2,5) the coefficient of variation was as large as 6%. Control results showed an average oxygen uptake rate of 7,2% per min.

Results on the effect of salt concentration (Table 2), hardness (Table 3), and pH (Table 4) on oxygen uptake rate of *T. pyriformis* showed that a twentyfold concentrate of Osterhout salt solution, 'hard' water and pH values 4 to 8 (corresponding respectively with salt concentration, hardness and pH of the wastewater samples) all gave a similar response to that of the control tests (oxygen uptake rate of 7,2% per min). Test oxygen uptake rates (wastewater effluent samples) significantly in excess of or less than 7,2% per min (at P = 0,05) were thus attributed to toxic activity in the sample solution.

The effect of the various wastewater effluent samples on the oxygen uptake rate of *T. pyriformis* is illustrated in Figures 2 to 4. With the exception of sample C3(1980-10-27 and 1980-11-28) all test samples affected respiration rate (indicating toxic activity). As might be expected, the raw and settled wastewater effluent samples (A1, A2, B1, C1) generally exhibited a marked toxic effect on the oxygen uptake rate of *T. pyriformis*, the less polluted effluents showing considerably less toxic activity. The raw and settled wastewater effluents varied in toxicity from one sample to another while humus tank, biofilter, activated sludge and denitrification plant effluents exhibited more or less the same toxicity. The industrial effluent (A1) collected on 1980-11-07 produced a lethal effect.

Toxicity of complex water samples could be attributed to the

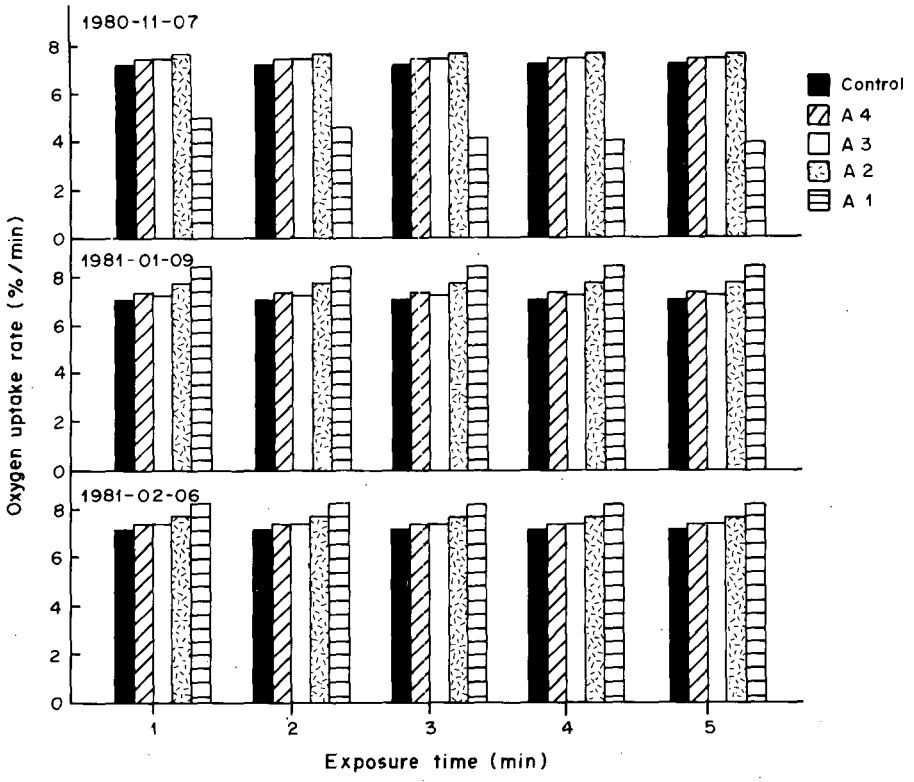

Figure 2
Effect of samples from wastewater treatment plant A on the oxygen uptake rate of T. pyriformis. *Results are expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions*

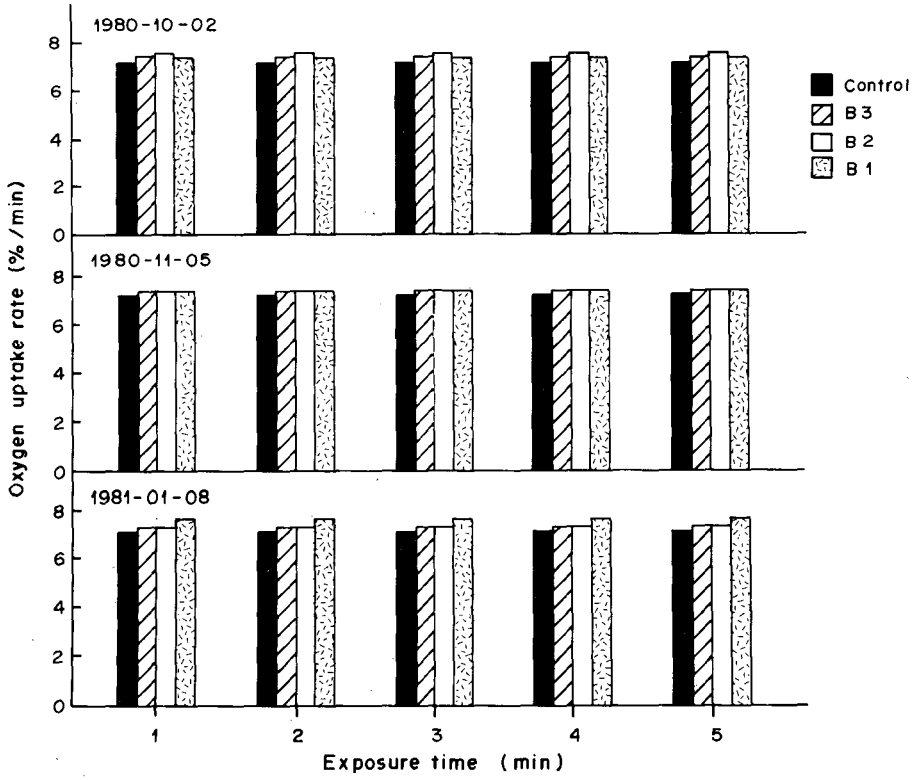

Figure 3
Effect of samples from wastewater treatment plant B on the oxygen uptake rate of T. pyriformis. *Results are expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions*

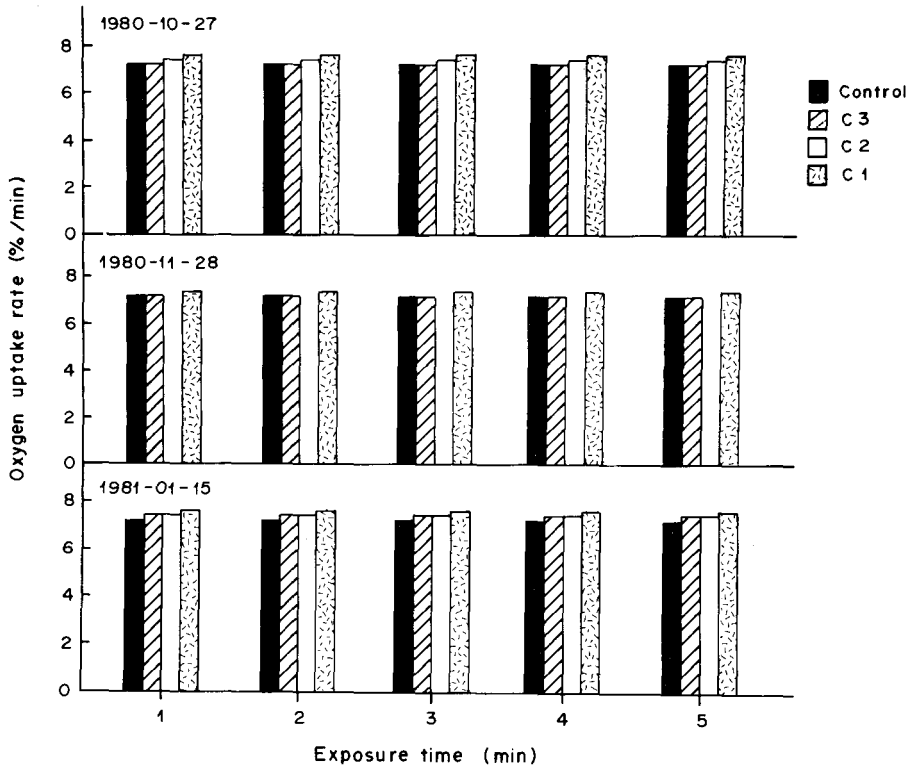

Figure 4
Effect of samples from wastewater treatment plant C on the oxygen uptake rate of T. pyriformis. *Results are expressed in relation to a reference oxygen uptake rate of 8% per min. Each result is an average of six repetitions*

TABLE 5
ANALYTICAL RESULTS OF SAMPLES FROM WASTEWATER TREATMENT PLANT A

	Date	1980-11-07				1981-01-09				1981-02-06			
	Sample No.	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3	A4
	pH	5,9	7,6	7,6	7,6	6,6	7,2	8,2	7,8	6,1	7,3	8,0	7,6
mg/ℓ	Sodium (Na)	124	78	112	114	128	90	110	112	144	80	145	140
	Potassium (K)	23	19	22	22	22	19	20	19	20	18	20	19
	Calcium (Ca)	34	34	41	36	41	43	40	41	45	40	38	38
	Magnesium (Mg)	13	12	14	14	15	15	15	14	14	15	16	15
	Chloride (Cl)	106	52	61	63	73	60	71	73	73	60	75	70
	Sulphate (SO_4)	140	80	200	150	130	90	135	130	250	120	215	190
	Kjeldahl-N (N)	29,0	49,0	11,0	3,0	12,0	34,0	2,4	4,2	14,5	41,5	4,1	1,8
	Ammonia-N (N)	16,0	39,0	2,8	0,7	9,7	30,5	0,8	2,7	8,5	40,0	1,3	<0,2
	Methylene blue active substances (LAS)	8,0	10,1	1,4	1,6	4,4	6,4	1,4	1,1	2,6	5,1	1,1	1,0
	Chemical oxygen demand (COD)	700	260	100	80	510	220	85	75	655	190	80	42
	Total alkalinity ($CaCO_3$)	147	255	98	89	220	280	151	178	129	250	152	93
µg/ℓ	Aluminium (Al)	660	280	<100	<100	690	<100	<100	<100	550	220	100	<100
	Boron (B)	2 100	1 250	550	500	1 025	700	350	350	700	525	375	325
	Cadmium (Cd)	<5	<5	<5	<5	7	6	8	9	<5	<5	<5	<5
	Chromium (Cr)	210	<25	<25	<25	105	<25	<25	<25	250	<25	30	35
	Copper (Cu)	4 630	<25	40	40	440	30	65	30	330	<25	50	70
	Iron (Fe)	1 910	270	250	620	1 500	220	170	460	1 940	360	180	450
	Mercury (Hg)	<1	<1	1	3	2	2	4	5	12	2	2	1
	Lead (Pb)	50	<25	<25	<25	105	<25	<25	40	<25	<25	<25	<25
	Zinc (Zn)	520	90	60	50	280	120	65	70	280	80	55	45
	Phenols	355	140	90	105	400	145	120	20	290	300	25	20
	Cyanide (CN)	13 600	660	595	<50	600	510	<50	<50	50	320	500	<50

TABLE 6
ANALYTICAL RESULTS OF SAMPLES FROM WASTEWATER TREATMENT PLANT B

	Date	1980-10-02			1980-11-05			1981-01-08		
	Sample No.	B1	B2	B3	B1	B2	B3	B1	B2	B3
	pH	8,0	8,0	8,0	7,8	8,1	7,9	7,4	8,0	8,1
mg/ℓ	Sodium (Na)	68	67	64	61	62	63	66	71	65
	Potassium (K)	16	15	16	13	13	15	16	16	16
	Calcium (Ca)	43	41	40	38	37	37	54	47	49
	Magnesium (Mg)	20	20	22	19	19	20	21	24	22
	Chloride (Cl)	61	64	67	52	54	55	78	83	70
	Sulphate (SO_4)	75	83	81	56	69	68	74	70	90
	Kjeldahl-N (N)	26,0	11,0	7,3	29,0	7,2	8,3	27,0	11,0	7,9
	Ammonia-N (N)	24,0	6,5	7,0	20,0	4,4	5,2	25,0	8,0	6,6
	Methylene blue active substances (LAS)	7,5	1,7	1,6	5,4	1,2	1,4	3,2	1,3	1,1
	Chemical oxygen demand (COD)	210	44	43	148	41	42	180	70	70
	Total alkalinity ($CaCO_3$)	254	152	151	255	130	135	258	167	161
µg/ℓ	Aluminium (Al)	340	140	<100	310	<100	<100	240	<100	<100
	Boron (B)	700	250	270	1 100	280	400	450	200	200
	Cadmium (Cd)	<5	<5	<5	<5	<5	<5	<5	<5	<5
	Chromium (Cr)	<25	<25	<25	<25	<25	<25	<25	<25	<25
	Copper (Cu)	<25	<25	<25	<25	<25	<25	40	<25	<25
	Iron (Fe)	270	60	140	220	30	55	300	70	115
	Mercury (Hg)	15	<1	2	4	4	5	8	6	4
	Lead (Pb)	60	<25	<25	<25	<25	<25	<25	<25	<25
	Zinc (Zn)	170	30	45	80	35	30	155	65	75
	Phenols	172	89	55	190	140	125	50	15	80
	Cyanide (CN)	410	<50	<50	375	<50	<50	385	250	<50

TABLE 7
ANALYTICAL RESULTS OF SAMPLES FROM WASTEWATER TREATMENT PLANT C

	Date	1980-10-27			1980-11-28			1981-01-15		
	Sample No.	C1	C2	C3	C1	C2	C3	C1	C2	C3
	pH	7,3	7,4	7,5	7,9	7,7	7,8	7,3	7,5	7,8
mg/ℓ	Sodium (Na)	60	70	76	59	64	68	76	88	80
	Potassium (K)	14	16	16	14	14	15	18	15	14
	Calcium (Ca)	34	38	39	26	28	32	48	48	47
	Magnesium (Mg)	13	14	14	11	12	11	18	18	15
	Chloride (Cl)	53	50	63	40	40	45	63	60	65
	Sulphate (SO_4)	80	80	95	60	70	71	120	110	115
	Kjeldahl-N (N)	24,5	8,0	1,7	24,0	10,0	18,5	24,0	4,8	1,2
	Ammonia-N (N)	22,5	4,8	<0,2	14,5	5,2	6,5	9,8	3,6	<0,2
	Methylene blue active substances (LAS)	5,3	1,8	1,4	6,1	1,8	1,2	3,9	1,5	0,7
	Chemical oxygen demand (COD)	153	77	65	141	72	70	180	80	48
	Total alkalinity ($CaCO_3$)	200	50	85	185	50	184	203	60	128
µg/ℓ	Aluminium (Al)	420	130	110	240	<100	<100	<100	<100	<100
	Boron (B)	820	350	300	410	250	450	400	350	275
	Cadmium (Cd)	<5	<5	<5	<5	<5	<5	6	6	<5
	Chromium (Cr)	<25	<25	<25	<25	<25	<25	<25	<25	<25
	Copper (Cu)	<25	<25	<25	<25	<25	<25	30	<25	<25
	Iron (Fe)	160	60	<25	170	60	105	220	55	<25
	Mercury (Hg)	2	2	1	<1	<1	<1	2	<1	4
	Lead (Pb)	<25	<25	<25	<25	<25	<25	<25	30	<25
	Zinc (Zn)	240	250	130	350	100	60	480	340	45
	Phenols	62	23	12	100	20	20	35	12	80
	Cyanide (CN)	653	<50	<50	1 500	<50	<50	1 350	190	<50

total concentration of known and unknown chemical compounds present in the samples rather than to the presence of specific individual toxicants (Kingsbury and Rees, 1978; Cairns and Van der Schalie, 1980). Analytical results of the wastewater effluent samples (Tables 5, 6 and 7) show the presence of several contaminants which could have contributed to toxic activity in the sample solution. The marked differences in toxic effects between the raw and settled wastewater effluents and the other samples could be ascribed *inter alia* to the considerable reductions which took place in concentrations of such determinands as total Kjeldahl and ammonia nitrogen, chemical oxygen demand, aluminium, boron, zinc, phenols and cyanide during the various secondary treatment stages. Compared with the other wastewater effluent samples, industrial wastewater effluent (A1) contained relatively high concentrations of several metals, for example aluminium, chromium, copper and iron, all of which could have contributed to the changed oxygen uptake rates produced by these samples.

The addition of test samples to cell suspensions involves a twentyfold dilution in sample concentration. With the exception of detectable concentrations of cyanide in raw and settled wastewater effluents and in humus tank effluent from wastewater treatment plant A, all chemical compounds in wastewater effluent samples were present in concentrations much lower than the established lowest individual toxicant concentrations affecting a response in *T. pyriformis* (Slabbert and Morgan, 1982). The lethal effect of the industrial effluent (A1) collected on 1980-11-07 could, to a great extent, be attributed to the extremely high cyanide concentration (0,68 mg/ℓ after addition to cells) in the test sample. Individual toxicity tests showed (Slabbert and Morgan, 1982) that cyanide decreased oxygen uptake rate to a greater or lesser extent, depending on the concentration employed, in much the same way as the industrial effluent sample. A concentration of 0,5 mg/ℓ cyanide caused an immediate and almost total inhibition of oxygen uptake rate (Slabbert, 1982). After the twentyfold dilution all the wastewater effluent samples (with the exception of A1, 1980-11-07, which contained 0,68 mg/ℓ cyanide) contained individual determinands in concentrations much lower than those permitted in terms of the general standard for industrial effluents discharged into receiving waters (South Africa, 1962).

Conclusions

Decontamination of samples is a necessary step in the bioevaluation of heavily contaminated water samples in order to interpret toxic effects on oxygen uptake rate.

The total salt concentration and pH of wastewater effluent samples had no effect on the oxygen uptake rate of the test organism, consequently test uptake rates in excess of or less than the control rate (7,2% per min) were attributed to toxic activity in the sample solution.

A comparison of toxic effects with the concentration of individual chemical substances present after addition of the test samples to cell suspensions, showed that the effect of wastewater effluents on respiration rate of the test organism could be attributed to the overall chemical activity (synergistic and antagonistic) rather than to the presence of specific individual toxicants.

The *T. pyriformis* bioassay system proved to be sufficiently sensitive for rapid routine monitoring of raw and treated wastewater effluents and their quality control (within the limits set for industrial effluents) if discharged into receiving waters. Because of relatively large differences in toxic effects between raw and treated effluents the technique may be successfully applied to evaluate the efficiency of secondary treatment processes for the removal of toxicants.

Acknowledgements

The assistance of the staff of the Water Quality Division of the National Institute for Water Research, who carried out the chemical analyses, is gratefully acknowledged.

This paper is published with the permission of the Director of the National Institute for Water Research.

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