A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants

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Abstract

This study presents a closed-system algal toxicity test technique that is capable of detecting the effects of both organic and metallic toxicants. Toxicity testing was conducted by transferring adequate amounts of algal suspension, dilution water (with culture growth medium), and toxicants into 300-mL BOD bottles. The BOD bottles were completely filled up with no head-space left. The initial cell density and the exposure time were 15,000 cells/mL and 48 h, respectively. The performance of the above test method was evaluated using three heavy metals and six organic toxicants based on three different test endpoints, i.e., dissolved oxygen production, algal growth rate, and cell density. The proposed test revealed excellent test sensitivity and reproducibility. Currently, none of the existing algal toxicity test protocols is adequate for assessing the toxicity of organic chemicals. The closed-system algal toxicity tests developed by previous researchers also may not be ideal because the enlarged headspace and/or enriched bicarbonate buffer may result in either underestimations of the exposure concentrations or insensitive responses to both heavy metals and organic toxicants. Compared to the aforementioned algal toxicity test methods, the proposed technique in the present study has a more general applicability under conditions such as effluent samples containing both metals and organic toxicants or samples with unknown compositions.
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1. Introduction

Algae are ecologically important organisms in the aquatic food chain and are frequently used in environmental studies for assessing the relative toxicity of various chemicals and/or waste discharges. Currently, batch technique is adopted by most standard algal test protocols for regulatory purposes (ASTM, 1994; ISO, 1987; US EPA, 1996; OECD, 1984). Those methods, although employing different medium compositions, are basically open-system tests because the major carbon source for algal growth is from the atmospheric air. Previous studies showed that algal toxicity tests were relatively insensitive to organic toxicants as compared to other toxicity tests based on organisms such as fish, daphnia or luminescent bacteria (Munkittrick et al., 1991; Toussaint et al., 1995; Peterson and Peterson, 1996). The main reason causing the above phenomenon can be related to the open test environment and vigorous mixing usually employed by the batch-type algal toxicity tests. Such experimental design causes the loss of volatile organic toxicants and, consequently, underestimations of the toxicity of volatile organic chemicals. The European Centre for Ecotoxicology and Toxicology of Chemicals (1996) has concluded that current algal toxicity test protocols are unsuitable for assessing the effects of volatile compounds.

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Closed vessels with headspace have been applied in several studies to minimize losses of volatile toxicants (Herman et al., 1990; Brack and Rottler, 1994; Galassi and Vighi, 1981). Under closed-test environment, a major consideration in the experimental design was to avoid CO_2 deficiency. Sufficient carbon supply was achieved by either providing a large enough headspace volume together with enriched carbon dioxide gas or adding surplus amount of carbonates/bicarbonates into the growth medium (Galassi and Vighi, 1981; Herman et al., 1990; Mayer et al., 2000). Large headspace may cause a significant portion of the volatile compound to partition from the aqueous phase into the headspace until equilibrium is reached. Mayer et al. (2000) pointed out that the exposure concentration may thus be altered significantly. Therefore, they proposed the use of a closed-test system with no headspace and an enriched bicarbonate buffer in the growth medium (300 mg/L of $NaHCO_3$). The effects of enriched buffer on toxic responses and the test alga, however, have not been carefully studied by previous researchers. Brack and Rottler (1994) pointed out that enriched carbonate buffer may result in increased ionic strength and lower test sensitivity. To avoid drastic changes in ionic strength, in their study, they used a two-compartment test system (bipartite culture flasks) to separate the carbonate buffer from the growth medium. Following the above development, sealed exposure system and sufficient CO_2 supply were suggested by the International Organization for Standardization and the Organization for Economic Co-operation and Development for testing volatile compounds (ISO, 1998; OECD, 2000).

The conventional response endpoints applied in algal toxicity test include final yield (biomass or cell densities), growth rate, chlorophyll contents and total biovolume. Analysis of experimental results from International Standards Organization's ring tests (Hanstveit and Old-ersma, 1981; Hanstveit, 1982) show that EC_{50} values based on final yield (biomass) were generally lower and could differ by a factor of 2 compared with those based on growth rate. Nevertheless, algal growth rate has been considered as a more meaningful and consistent parameter than total cell number or biomass as expressed, e.g., by cell volume. Toxicity data based on growth rate were found to provide greater reproducibility and, hence, better comparability for test results from different laboratories (Nyholm, 1985; OECD, 2000). In the past decade, ^{14}C assimilation and oxygen production have been used as response endpoints for assessing the toxic effects of chemicals on algal photosynthesis. Nyholm and Damgaard (1990) considered the ^{14}C method as less sensitive than the conventional response parameters. However, Pardos et al. (1998) reported that this method is relatively more sensitive than the traditional batch tests. The oxygen production method has been found to be less sensitive than the ^{14}C method (Versteeg, 1990). The major advantage for the above two test methods is the shorter exposure time required which varies from 30 min to less than 1 day. On the other hand, a common disadvantage of both methods is the requirement of sophisticated equipments such as the liquid scintillation counter and the respirometer.

Current algal toxicity protocols are inadequate for testing volatile organic chemicals. On the other hand, the aforementioned closed-system test methods also may not be ideal for testing heavy metals due to possible complexation effects caused by the enriched carbonate buffer. The aim of this paper is to present a simple and low-cost algal toxicity test that can be applied to the assessment of both metallic and organic toxicants. This study also attempted to evaluate the necessity of enriched carbonate buffer.

2. Materials and methods

2.1. Algal incubation

The alga *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*, UTEX 1648) was grown in a 4-L transparent chemostat incubator. The growth medium was continuously supplied by a variable-speed

Table 1
Composition of culture medium for algae

Compounds	Concentrations (mg/L)	Compounds	Concentrations (µg/L)
$NaNO_3$	12.75[a] (25.5[b])	H_3BO_3	186
$NaHCO_3$	15.0	$MnCl_2 \cdot 4H_2O$	415.6
K_2HPO_4	0.52[a] (1.04[b])	$ZnCl_2$	3.27
$MgSO_4 \cdot 7H_2O$	14.7	$CoCl_2 \cdot 6H_2O$	1.428
$MgCl_2 \cdot 6H_2O$	12.16	$CuCl_2 \cdot 2H_2O$	0.012
$CaCl_2 \cdot 2H_2O$	4.41	$Na_2MoO_4 \cdot 2H_2O$	7.26
		$FeCl_3 \cdot 6H_2O$	160.0
		$Na_2EDTA \cdot 2H_2O$	30[a] (0[b])

[a]Concentration applied to chemostat incubation.
[b]Concentration applied to toxicity testing.

pump (flow rate = 1 L/day). Air agitation was used to achieve adequate mixing. The chemostat reactors were placed in a constant-temperature room at $24 \pm 1\,°C$. Light intensity was set at $65\,\mu E\,m^{-2}\,s^{-1}$ ($\pm 10\%$). Culture medium composition is listed in Table 1 and is basically the same as that described by the EPA bottle technique (US EPA, 1996). However, according to our previous work (Chen and Lin, 1997), $NaNO_3$, K_2HPO_4, and EDTA contents were reduced to 12.75, 0.52 mg/L, and $30\,\mu g/L$, respectively. The dilution rate (D) for the chemostat was set at 0.25/day to ensure a nutrient-limited condition. Quality assurance (QA) procedures were routinely conducted by plotting control charts of cell density and pH to verify that steady state was achieved and well maintained. A detailed description regarding the chemostat incubation can be found from the author's previous work (Chen and Lin, 1997).

2.2. Toxicity testing

After the algal incubator has reached the steady state, toxicity testing was conducted by transferring adequate amounts of algal suspension, dilution water (with growth medium), and toxicants into 300-mL BOD bottles. The BOD bottles were completely filled up with no head space left. Water seal was provided to ensure a closed-test environment. The bottles were then placed on an orbital shaker (Ferstek, Model S103) operated at 100 rpm. Temperature and light intensity were kept the same as the algal incubator. US EPA (1996) bottle medium with no ethylenediaminetetraacetic acid (EDTA) content was used for toxicity testing. The dilution water was stripped by nitrogen gas to reduce the initial dissolved oxygen level to approximately 1–3 mg/ L. In addition, the N_2 gas contained 0.5% carbon dioxide as an extra carbon source. The initial pH for the growth medium was 7.5. Three response endpoints were used to evaluate the toxicity of toxicants; dissolved oxygen production (ΔDO), the delta cell density, and the algal growth rate based on cell density. The median effective concentration (EC_{50}) was defined as the toxicant concentration that resulted in 50% reduction with respect to the response endpoints (i.e., ΔDO, $\Delta cell$ density and growth rate). In a preliminary study, test conditions (in terms of the initial cell densities and test
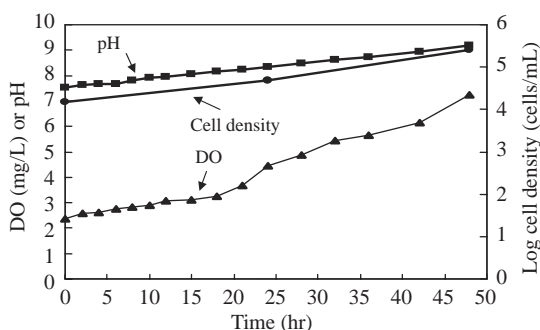

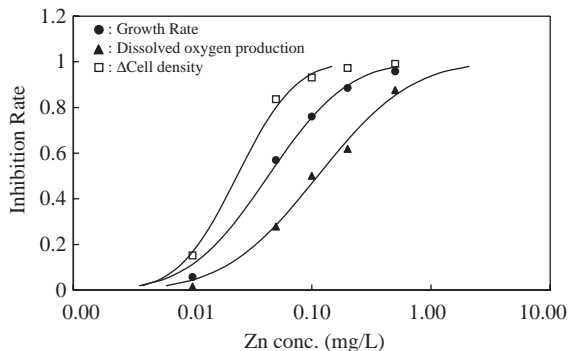Fig. 1. The variation of pH, cell density, and DO in a test control.

Fig. 2. The concentration–response curves of algal toxicity tests with respect to zinc.

Table 2
Algal responses in terms of DO production and cell density

Zn (mg/L)	Initial DO (mg/L)	Final DO (mg/L)	Final cell density (cells/mL)	DO (mg/L)	Cell density	Specific growth rate (μ)	Inhibition rate		
							DO	Cell density	Growth rate
Control	2.02	7.51	206,900[a]	5.49	191,900	1.312	0	0	0
0.5	2.70	3.37	16,733	0.68	1733	0.0538	0.876	0.990	0.958
0.2	2.35	4.44	20,266	2.09	5266	0.150	0.619	0.972	0.885
0.1	2.17	4.91	28,133	2.74	13,133	0.314	0.500	0.931	0.760
0.05	2.12	6.08	46,400	3.96	31,400	0.562	0.278	0.836	0.569
0.01	2.04	7.44	177,800	5.40	162,800	1.236	0.016	0.151	0.057
EC50							0.112	0.024	0.0432

[a]Initial cell density = 15,000 cells/mL.

duration) were optimized through a series of trial-and-error tests. The objective of these trials was to achieve maximum test sensitivity and, yet, maintain satisfactory reproducibility. In general, lower cell densities resulted in higher sensitivity with poorer test repeatability. Furthermore, longer exposure time may increase the test sensitivity but, in some instance, result in insufficient carbon supply due to the excessive production of algal biomass. The optimum inoculated cell density and the test duration were 15,000 cells/mL and 48 h, respectively.

Toxicant concentrations presented in this work are in the form of nominal concentration. Concentration controls were periodically conducted following exactly the same procedure as described above. The only difference is that no algal inoculum was added to the concentration controls. The prepared controls were then analyzed using an atomic absorption spectrophotometer (Hitachi, Model Z-8100) or a HPLC analyzer (Waters, 2996 Photodiode Array Detector). The difference between the nominal and measured concentrations was found to be less than 6%. All chemicals used were of reagent grade. All tests were performed in triplicate.

3. Results and discussion

Fig. 1 shows the variation of pH, cell density, and DO in a test control. The pH value increased from 7.5 to 9.17, while the cell density was raised from 15,000 cells/mL to a final yield of 252,900 cells/mL. Exponential growth was observed during the exposure period. The specific growth rate (μ) of the control was 1.41/day. Due to the variation of light intensities ($\pm 10\%$) on the shaker panel and other inexplicable reasons, the specific growth rate for the controls varied from 1.2/day to 1.5/day and was somewhat lower than that observed (1.4–1.8/day) from the conventional batch technique (Halling-Sørensen et al., 1996). This is probably due to the fact that, in a closed-test system without headspace, the only carbon source for algal growth is from the conversion of bicarbonates to CO_2. The dissolved oxygen content, on the other hand, increased from an initial level of 2.37–7.23 mg/L.

The carbon consumption for algal growth can thus be calculated using the following equations:

$$DWT_{algal} = m_{algal} \cdot \Delta Cell\ density, \tag{1}$$

$$U_{CO_2,algal} = \frac{m_{c,algal}\ DWT_{algal}\ M_{CO_2}}{M_C}, \tag{2}$$

where $U_{CO_2,algal}$ is the consumption of CO_2 for algal growth, DWT_{algal} the increased dry weight algal biomass during the test period in milligrams, $m_{c,algal}$ the mg carbon/mg DWT_{algal} (usually 50%, Goldman et al., 1974), m_{algal} the mg DWT_{algal}/cell number (mg dry

Table 3
Performance of the BOD bottle test

Method	BOD bottle			Continuous	Batch	
Metal	EC$_{50}$ (mg/L)			EC$_{50}^a$ (mg/L)	EC$_{50}^b$ (mg/L)	EC$_{50}^c$ (mg/L)
	Growth rate	Cell density	DO			
Zn	0.043 [3]	0.024 [2]	0.112 [5]	0.015 [1]	0.178 [6]	0.06 [4]
Pb	0.252 [2]	0.085 [1]	0.472 [4]	0.256 [3]	2.655 [6]	0.5 [5]
Cd	0.035 [3]	0.025 [2]	0.080 [5]	0.013 [1]	0.341 [6]	0.04 [4]
Ave. ranking	2.67	1.67	4.67	1.67	6	4.33

[ ]: Ranking value.
[a] Data from Chen and Lin (1997), based on algal growth rate.
[b] Data from Chen and Lin (1997), based on algal growth rate.
[c] Data from Healey and Hendzel (1975), Blaylock et al. (1985), based on algal biomass.

weight per cell), M_{CO_2} the molecular weight of CO_2, M_C the molecular weight of C.

In Eq. (1), Δcell density is equal to 237,900 cells/mL and m_{algal} has been determined experimentally as 1.58×10^{-9} mg/cell. Using Eq. (2), carbon consumption for the control can thus be determined as 0.689 mg/L (or 0.0157 mM HCO_3^- equivalent). Since exponential growth can be maintained during the 48 h test period and the amount of $NaHCO_3$ in growth medium is 15 mg/L (0.179 mM), we concluded that the bicarbonate content in US EPA bottle medium is sufficient for supporting algal growth. The conversion of bicarbonates to CO_2 may, however, result in higher pH (9.17) level at the end of the test.

Table 2 displays a typical set of algal responses with respect to the toxicity of zinc. For the test control, the dissolved oxygen concentration increased from 2.02 mg/L at the beginning to a final DO concentration of 5.49 mg/L. The cell density increased from an initial value of 15,000 cells/mL to a final yield of 206,900 cells/mL. At a specific zinc concentration, we may find that inhibition rate based on delta cell density is always greater than that based on delta DO. We may thus conclude that cell density is a more sensitive parameter than DO. The sensitivity of the growth-rate endpoint is lower than that based on cell density, but is better than

DO production. Concentration response curves for the aforementioned response endpoints are shown in Fig. 2. These curves were obtained through linear regression assuming a log-normal distribution (probit model) of the tolerances. Based on the probit analyses, EC_{50} values were found to be equal to 0.024 mg/L (Δcell density), 0.043 mg/L (growth rate) and 0.112 mg/L (ΔDO). Response endpoint based on cell density is approximately five times more sensitive than DO production.

The performance of the above BOD-bottle technique was evaluated using three heavy metals (Zn, Pb, and Cd) as listed in Table 3. The EC_{50} values derived from continuous and batch algal toxicity tests are also given in Table 3, for comparison. The BOD-bottle test is less sensitive than the continuous test developed previously (Chen and Lin, 1997). The main reason is that, for the BOD-bottle test, algae were cultured under nutrient-saturated conditions. On the other hand, the continuous tests were conducted under nutrient-limited conditions. Our previous studies verified that algae subjected to phosphorus and/or nitrogen limitations will become more sensitive (Chen, 1994; Chen and Lin, 1997). The new technique is more superior to the batch test
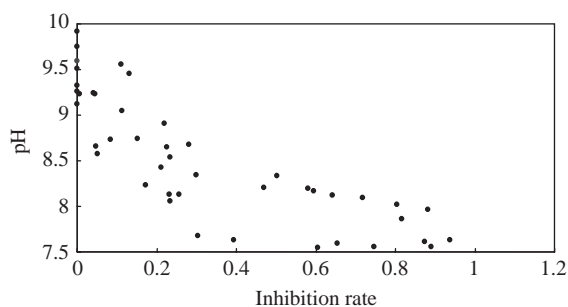

Fig. 3. pH variation vs. inhibition rate (based on Δcell density).
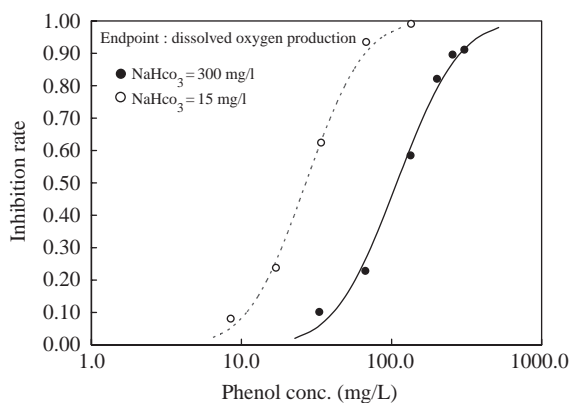

Fig. 4. Dose–response curves of phenol under different bicarbonate concentrations.

Table 4
Effective concentrations (mg/L) with respect to normal and enriched bicarbonate buffers

Endpoint	EC value	(a) Phenol (normal)[b]	(b) Phenol (enriched)[c]	(b)/(a) ratio	(a) Zinc (normal)	(b) Zinc (enriched)	(b)/(a) ratio
DO	EC_{50}	26.85	108.3	4.0	0.112	0.377	3.37
	EC_{10}	10.82	40.83	3.77	0.0181	0.0558	3.25
Density	EC_{50}	10.88	105.3	9.68	0.0236	0.0853	3.61
	EC_{10}	3.89	33.12	8.51	0.0075	0.0191	2.53
GR[a]	EC_{50}	19.14	224.7	11.7	0.0432	0.329	7.62
	EC_{10}	4.06	63.04	15.5	0.0092	0.0171	1.87

[a]Growth rate.
[b]$NaHCO_3$ concentration = 15 mg/L.
[c]$NaHCO_3$ concentration = 300 mg/L.

considering algal growth rate or cell density as the response endpoints. In Table 3, a ranking system was used to compare the relative sensitivities among various test methods and endpoints. For each specific metallic toxicant, the most sensitive test endpoint or method was assigned with the smallest numerical value for ranking, e.g., (ASTM, 1994). Larger ranking values were then given to the endpoints or methods that are less sensitive, accordingly. From the average ranking values, we may conclude that the order of test sensitivity is as follows: continuous test = BOD bottle test (Δcell density) > BOD bottle test (growth rate) > batch test (biomass) > BOD bottle test (DO production) > batch test (growth rate).

Current algal toxicity test protocols impose strict restrictions on pH variation to avoid drastic changes of the concentrations of dissolved metallic species in the solution. For example, OECD specifies that pH variation during the exposure period should be less than 1 pH unit (OECD, 1984, 2000). Similarly, the International Organization for Standardization also requires that changes should be kept within 1.5 pH units (ISO, 1987). The proposed test technique did not increase the bicarbonate concentration in the culture medium to improve its buffering capacity. Hence, pH variation for the controls is slightly greater than 1.5 units (Fig. 1). However, comparison in Table 3 shows that our test method still reveals superior test sensitivity as compared to conventional batch tests. Fig. 3 depicts the relationship between pH changes and inhibition rates recorded from some tests conducted in this study. One may find that, if a treatment exerts noticeable inhibitory effect (e.g., greater than or equal to 20%) on algal growth, the final pH change will always be less than 1.5 units. In other words, concentrations of dissolved metallic species for most treatments may not be altered significantly due to pH changes. The inhibitory effects in Fig. 3 were calculated based on the endpoint of Δcell density. Similar observations were obtained when the inhibition

rates were estimated based on DO production or growth rate.

Enriched bicarbonate buffer has been used to reduce pH variation by several researchers in their experimental design for closed-system toxicity tests (Galassi and Vighi, 1981; Herman et al., 1990; Mayer et al., 2000). Table 4 lists the EC_{50} and EC_{10} values for phenol and zinc obtained from two series of toxicity tests with $NaHCO_3$ concentration equal to 15 mg/L (normal) and 300 mg/L (enriched), respectively. For phenol, both EC_{50} and EC_{10} increased approximately 4 to 15 times when enriched bicarbonate buffer was applied. Similar phenomena were observed for the zinc toxicant, where EC_{50} and EC_{10} increased 2 to 7 times compared to the results based on normal growth medium composition. Fig. 4 depicts the dose–response curves for phenol according to the inhibitory effects on DO production. We may find that the entire dose–response curve shifted to the right when the bicarbonate concentration was increased. Therefore, it is obvious that enriched bicarbonate buffer may drastically reduce the test sensitivity and result in extremely large EC_{50} and EC_{10} values. The addition of surplus bicarbonate buffer has been suggested by the OECD Guidance as one of the alternatives to minimize pH variation (OECD, 2000). The results in Table 4, however, suggest that such an approach may not be adequate.

EC_{50} values derived from various O_2 production tests and the ^{14}C assimilation tests were compared and listed in Table 5. With respect to DO-production endpoint, the new proposed test shows better sensitivity compared to other tests based on oxygen production (Turbak et al., 1986; Versteeg, 1990) and performs equally well as the ^{14}C assimilation tests. Considering the cell density as the test endpoint, our new method is clearly more superior to all the other test methods listed in Table 5. The reasons for the different sensitivities revealed by various test methods are quite complicated and can be related to

Table 5

Comparisons with the O_2 production and the ^{14}C assimilation methods

Data source	Zn	Cd	Test condition
This study (Growth rate)	0.0432	0.0347	1.5×10^4 cells/mL, 48 h
(ΔCell density)	0.024	0.0256	1.5×10^4 cells/mL, 48 h
(DO production)	0.112	0.080	1.5×10^4 cells/mL, 48 h
O_2 Production			
Turbak et al. (1986)	0.178	0.13	$2-5 \times 10^5$ cells/mL, 24 h
Versteeg (1990)		4.7	10^6 cells/mL, 30 min
^{14}C Assimilation			
Versteeg (1990)		0.18	10^6 cells/mL, 24 h
Heever and Grobbelaar (1996)		0.071	10^4 cells/mL, 1 h
		0.077	10^4 cells/mL, 4 h
Pardos et al. (1998)	0.097	0.6	10^5 cells/mL, 4 h
	0.096	0.118	10^5 cells/mL, 48 h

the differences of followings: test endpoint, initial density of the inoculum, exposure time, and the sources of inoculated alga.

Six organic toxicants, as listed in Table 6, were tested and compared with literature data derived by various toxicity tests. The same ranking system described previously (Table 3) is applied herein to compare the relative sensitivities for various test organisms and endpoints. According to the average ranking values, the order of the relative sensitivity is as follows: rainbow trout > algae (cell density) > Daphnia magna > algae (growth rate) > algae (DO production) > Microtox > fathead minnow. The impression based on previous studies (Munkittrick et al., 1991; Toussaint et al., 1995; Peterson and Peterson, 1996) that algal toxicity tests are insensitive to organic toxicants, therefore, may not be adequate.

In Table 6, one may find that toluene, chlorobenzene, and benzene appeared to be very toxic to algal photosynthesis reactions judging from the ranking values for DO production endpoint. Kong et al. (1998) reported that toluene and malononitrile could significantly inhibit the activity of a photosynthesis enzyme (G6PDH). Our results are consistent with the above finding made by Kong et al. (1998). Previously, toxicity on algal growth and photosynthesis has rarely been simultaneously evaluated by algal toxicity tests. For the proposed test technique, the use of two (or three) different endpoints for the analyses of the toxic effects of organic toxicants can thus provide more information regarding the possible impact of organic chemicals.

The reproducibility of the proposed technique was evaluated using zinc and benzene. Table 7 shows that the coefficients of variation (CV) for growth rate and DO endpoints are approximately 10%. Delta cell density is the most sensitive response parameter but is also the least stable endpoint. The CV values for cell density are about 20% and are twice as much as that observed from DO and growth rate. The conventional batch test generally required 3 to 4 days to finish one test run and its intralaboratory precision ranged from 20 to 32% (Blaise et al., 1986; Van Coillie et al., 1982). Our new proposed method, on the other hand, has the advantages of shorter exposure time (48 h) and better test repeatability. Furthermore, based on previous studies (Vasseur and Pandard, 1988; Stratton and Gilles, 1990), the test sensitivity for the proposed technique can be significantly improved if the inoculated cell density is reduced to 10^3 cells/mL level. However we felt that, for regulatory purposes, consistent test results (reproducibility) should be a more important consideration than sensitivity. Hence, during the preliminary-study stage, the inoculum density was chosen at 15,000 cells/mL so that, CV values were kept below or equal to 20% for all response endpoints.

Table 6
EC50a values (mg/L) of organic toxicants derived from various test methods

Toxicant	Algae (BOD bottle) (48 h)			Microtox[b] (15 min)	Daphnia magna[b] (24 h)	Rainbow trout[b] (96 h)	Fathead minnow[b] (96 h)
	Growth rate	ΔCell density	ΔDO				
Phenol	19.14 [3]	10.88 [2]	26.85 [5]	21.00 [4]	32 [6]	9.9 [1]	34 [7]
2-CP[a]	13.01 [4]	8.63 [2]	20.55 [6]	39.71 [7]	18 [5]	2.62 [1]	11 [3]
2,4-DCP[a]	3.82 [4]	2.42 [1]	4.20 [5]	5.04 [6]	2.7 [3]	2.58 [2]	6.7 [7]
Toluene	21.60 [3]	14.81 [2]	16.55 [3]	19.70 [3]	15–23 [3]	8 [1]	34 [7]
Chlorobenzene	11.88 [4]	7.83 [2]	9.80 [3]	15.54 [5]	6.19 [1]	—	—
Benzene	28.70 [5]	16.46 [1]	20.86 [2]	35.70 [6]	22.53 [3]	—	25.28 [4]
Ave. ranking	3.83	1.67	4	5.17	3.5	1.25	5.6

[ ]: Ranking value.
[a]2-CP: 2-chlorophenol, 2,4-DCP: 2,4-dichlorophenol.
[b]Data from Walker (1988), Blum and Speece (1991), Chen and Yeh (1996), Ribo and Kaiser (1987), Shannon et al. (1991), Munkittrick et al. (1991), Zaho et al. (1998).

Table 7
Values of coefficients of variation (CV) calculated based on different endpoints

Endpoint	Growth rate		Cell density		DO	
Toxicant	EC50 (mg/L)	C.V.	EC50 (mg/L)	C.V.	EC50 (mg/L)	C.V.
Zn	0.0432 0.055 0.0463	10.37%	0.0236 0.0201 0.015	18.05%	0.112 0.092 0.101	8.04%
Average	0.048		0.0196		0.102	

Endpoint	Growth rate		Cell density		DO	
Toxicant	EC50 (mg/L)	C.V.	EC50 (mg/L)	C.V.	EC50 (mg/L)	C.V.
Benzene	31.52 30.88 27.49 28.46 25.17	9.00%	20.64 19.14 15.66 15.77 11.68	20.2%	19.23 20.69 23.27 23.02 18.11	10.91%
Average	28.70		16.46		20.86	

4. Conclusion

This study presents an algal toxicity testing technique which is capable of detecting the effects of both organic and metallic toxicants. The proposed test method revealed excellent test sensitivity and reproducibility. Furthermore, this test method has other advantages including shorter test duration and simple experimental design. Although enriched bicarbonate buffer was frequently applied in previous experiment designs for closed-system algal toxicity tests, the results from the present study show that such an approach is not adequate because the test sensitivity was drastically reduced due to the effects of high concentrations of buffer. In the author's viewpoint, the restrictions on pH variation imposed by many algal test protocols should not be applied to test controls and treatments with very low degrees of inhibition considering their minor influences on EC_{50} estimation. Currently, none of the existing algal toxicity test protocols is adequate for assessing the toxicity of organic chemicals. The closed-system algal toxicity tests developed by previous researchers also may not be ideal because the enlarged headspace and/or enriched bicarbonate buffer may result in either underestimations of the exposure concentration or insensitive responses to both heavy metals and organic toxicants. Compared to the afore-mentioned algal toxicity test methods, the proposed technique has a more general applicability under conditions such as effluent samples containing both metals and organic toxicants or samples with unknown compositions.

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