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A MULTI-CHANNEL CONTINUOUS WATER TOXICITY MONITORING SYSTEM: ITS EVALUATION AND APPLICATION TO WATER DISCHARGED FROM A POWER PLANT

BYOUNG CHAN KIM and MAN BOCK GU*
Advanced Environmental Monitoring Research Center (ADEMRC) and National Research Laboratory on Environmental Biotechnology, Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 1, Oryong-dong, Puk-gu, Gwangju, Republic of Korea
*(*author for correspondence, e-mail: mbgu@gist.ac.kr)*

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Abstract. A multi-channel continuous water toxicity monitoring system was, after confirming the systems' performance, implemented to samples of water discharged from power plants to detect and classify their toxicity using several recombinant bioluminescent bacteria. Each channel of the system is composed of a series of two mini-bioreactors to enable a continuous operation, i.e., without system interruption due to highly toxic samples. A different recombinant bacterial strain was present in each channel: DPD2540 (*fabA::lux CDABE*), DPD2794 (*recA::luxCDABE*), and TV1061 (*grpE::luxCDABE*), which are induced by cell membrane-, DNA-, and protein-damaging agents, respectively. GC2 (*lac::luxCDABE*) is a constitutive strain, whose bioluminescence is reduced by an increase in cellular toxicity. Phenol and mitomycin C (MMC) were used for evaluating the system's performance to detect toxic chemicals. These samples were injected into the second mini-bioreactor according to a step or bell-curve manner. The field samples used in this study were obtained from the water discharged from two different power plants in Korea – from a nuclear power plant and a thermo-electronic power plant, and were injected into the second mini-bioreactor to initiate the toxicity test. Each channel showed specific bioluminescent (BL) response profiles due to the toxic compounds present in the water samples. Comparing the BL signals between the standard toxic chemical samples and discharged water samples, the equivalent toxicity of the field water could be estimated. Finally, it was proved that this novel continuous toxicity monitoring system can be used as an alternative tool for the quick monitoring and control of water quality, as well as aid in the setting up of a new monitoring strategy to protect the source of tap water and in the prevention of polluted water discharge.

Keywords: bioluminescent bacteria, continuous toxicity monitoring, classification, discharged flow, multi-channel, power plants

1. Introduction

Most industrial plants are required to set-up and operate a wastewater treatment system (WWTS) according to the environmental protection policies of that nation. Water discharged from a WWTS should satisfy their guidelines, including the level of toxicity. Although most WWTS are well maintained, it is always necessary to regularly monitor the quality of the discharge flow. Furthermore, to prevent the

pollution of water resources by industries, it is important to remove contaminants before the treated water is discharged into the surface or ground waters. Sometimes, however, polluted water flows out without any treatment or the quality of the discharge does not satisfy the guidelines. This suggests that quality control, or the monitoring of water to be discharged, is essential in order to prevent the pollution of rivers and ground waters, which are used as sources of tap water. Protocols for the instrumental analysis of discharged water includes determining the COD, BOD, pH, organic matter, heavy metal concentration, and so on (Bitton and Dutka, 1986). Even though this gives quantitative data, they do not provide us information on the toxicity. Faster and more reliable toxicity tests are demanded as a result.

Toxicity tests for field water samples were initially performed using higher organisms like fish or *Daphnia magna*. These systems focused on changes in the organism's behavior, such as their movement patterns or population of death by analyzing these patterns using statistical methods (Kikuchi and Wakabayashi, 1997; Van der Schalie *et al.*, 2001). However, these approaches have some weak points. It is well known that both the fish and daphnia systems give us only the degree of toxicity, instead of that what damages triggered those behavior or death. Death or changes of behavior of test organisms do not furnish any information about what happens within the cells or about what factors are involved and how it occurs after exposure to test samples. As a result, these tests do not provide us with specific information in terms of the mode of toxic action of the samples. Also, these systems have to be stopped operation to re-initiate the system with fresh organisms if the system is exposed to highly toxic conditions, which means that it is not the real continuous monitoring. Finally, the detection time and reproducibility would be less reliable due to delayed response time of the organisms and the primitive analysis of the results. To overcome these weak points, recently, a novel multi-channel continuous toxicity monitoring system using genetically engineered bioluminescent bacteria (Lee *et al.*, 2003) has been developed and its system conditions optimized to monitor the toxicity of artificial water samples (Gu *et al.*, 1999; Gu and Gil, 2001), and some field samples (Gu *et al.*, 2001).

In this study, even though some of the characterization studies have been conducted previously (Gu and Gil, 2001), monitoring using the multi-channel continuous toxicity monitoring system was simulated with known toxic chemicals added as standards to the water samples to confirm that the strategy used to apply this system to real sites is plausible. The effects of different injection methods (step- and bell-shaped concentration curves) were also characterized. Based on these results, this system was next applied to determine the toxicity of water samples that was discharged from two power plants in Korea, and the monitoring and classification of the toxicity of each sample was been conducted based on a comparison between the signals from the samples and those obtained from spiked water samples.

2. Materials and Methods

2.1. The multi-channel continuous toxicity monitoring system and strains

The set up of the multi-channel continuous toxicity monitoring system followed the set up used previously for a single channel system (Gu *et al.*, 2001; Gu and Gil, 2001). Within each two-stage system, one of the bacterial strains, DPD2540, DPD2794, TV1061, or GC2, which harbor the plasmids *fabA::luxCDABE*, *recA::luxCDABE*, *grpE::luxCDABE*, and *lacZ::luxCDABE,* respectively, were cultured in the first reactor by the continuously feeding of medium into the first reactor. These recombinant bioluminescent strains show specific responses to membrane-damaging agents (DPD2540) (Choi and Gu, 2001), DNA-damaging agents (DPD2794) (Vollmer *et al.*, 1997), protein-damaging agents (TV1061) (Van Dyk *et al.*, 1995), or general cellular toxicity (GC2) (Gil *et al.*, 2000). The dilution rate of the first reactor was restricted to 0.8/h and that of the second reactor was 3.0/h. The specific operating conditions for each channel are listed in Figure 1. The system temperature was maintained at 30 °C for the DPD2540, DPD2794, and TV1061 channels and at 37 °C for the GC2 channel, as based on the previous studies with these strains. The pH of the medium was adjusted to 7.0 before autoclaving. Sterile distilled water was used when the reactors where not being run with a test sample. The outflows from the second reactor were gathered into a sterilized bottle and later sterilized before treated as normal wastes, and so no live bacterial cells are leaked in the real fields. The optical cell density was measured using UV spectrophotometer every 5 or 6 h under 600 nm. The cell optical density was maintained to 1.4∼1.5 in the second reactors during the continuous operation. Using a fiber optic probe connected with the second stage and a luminometer,

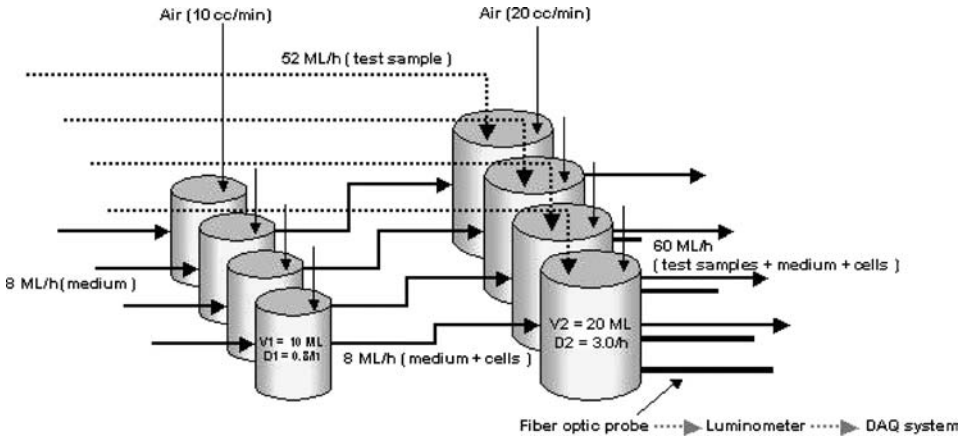

Figure 1. Operating conditions for the multi-channel continuous toxicity monitoring system.

changes in the bioluminescence were detected on-line using luminometer (TD-20, Turner, USA). All data is presented as the RBL values (Relative Bioluminescent Level = Test Bioluminescence level/Control Bioluminescence level). The unit of bioluminescence is an arbitrary unit from the luminometer.

2.2. CHEMICALS

Phenol and mitomycin C (MMC) were purchased from the Sigma Chemical Co. (U.S.A.). These chemicals were used to characterize the system. For the DPD2540, TV1061, and GC2 channels, phenol was used as a standard toxic chemical while MMC was used for the DPD2794 channel. These samples were injected into the second reactor to achieve a step or bell-shaped concentration curve.

2.3. FIELD SAMPLES

The field samples used in this study were obtained from two different discharges of separate power plants in Korea – from a nuclear power plant, referred to as site A, and from a thermo-electronic power plant, referred to as site B. The water was used as a coolant in the respective power plants. After taking a sample, using plastic bottles, they were immediately stored at 4 °C and used within 1 week. The pHs of each sample was about 7.0. These samples were injected into the second reactor according to a step-injection profile.

3. Results and Discussions

3.1. EVALUATION OF THE SYSTEM USING STANDARD TOXIC CHEMICALS

In real situations when pollutants leaked into aquatic environment, there might be a sudden increase in the chemicals or pollutants concentration, concomitant with an increase in the toxicity, or else certain chemicals are present in the discharged flow over a long period of time and disappeared suddenly or moderately. Taking these situations into consideration, toxic chemicals injection was simulated either using into the second mini-bioreactor of each channel either a step- or bell-shaped concentration profile. A step injection simulated sudden increase, maintenance, and sudden decrease of chemical concentration profiles and a bell-shaped injection simulated the profiles of the moderate increase and decrease of the chemical concentration. These injections were maintained for an appropriate amount of time to display the specific bioluminescent response of each channel.

Phenol is a well-known membrane and protein-damaging agent, and causes cellular damage, while MMC causes DNA damage (Choi and Gu, 2001; Vollmer *et al.*, 1997). Therefore, phenol and MMC were used as model toxic chemicals to evaluate and simulate the systems capability to monitor the toxicity of water

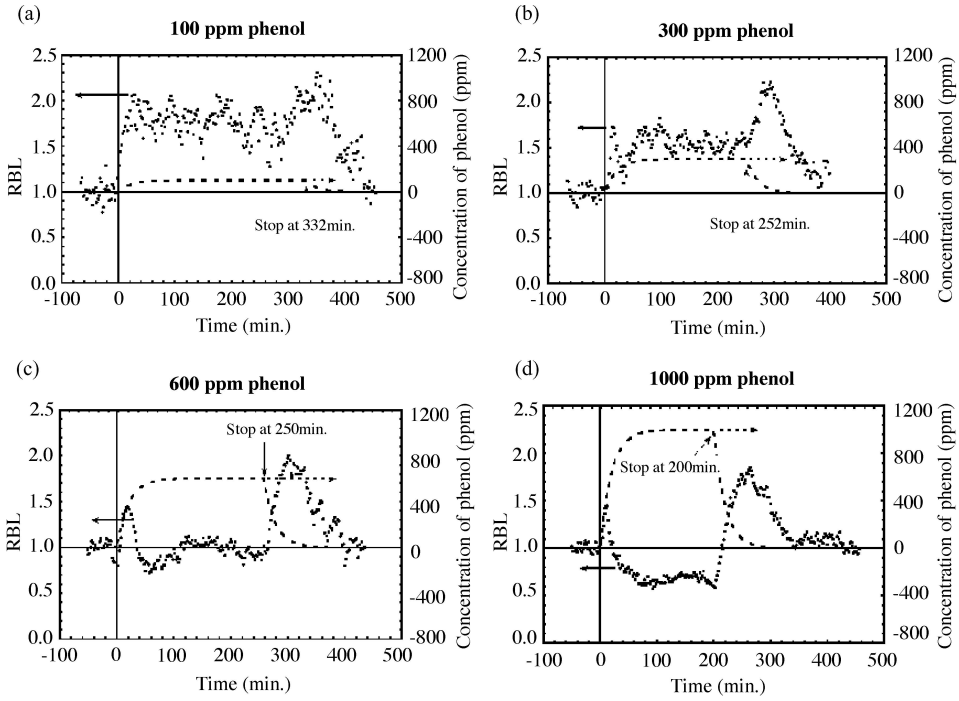

Figure 2. Responses of from DPD2540 to different phenol concentrations: (a) 100 ppm phenol, (b) 300 ppm phenol, (c) 600 ppm phenol, (d) 1000 ppm phenol. The dots are the measured bioluminescent values and the dashed lines are the concentration profiles in the 2nd reactor. A pulse-injection was used.

samples. As shown in Figures 2 and 3, specific signals were obtained from both the DPD2540 and GC2 channels to various phenol concentrations. Similarly, strains TV1061 and DPD2794 were also induced when exposed to phenol and MMC, respectively (data not shown). In the DPD2540 channel, after injection of phenol the RBL values increased suddenly. Additionally, when the phenol concentration was steady, the RBL values were also fairly consistent. During the tests with 600 ppm and 1000 ppm, however, the RBL values reached a maximum, decreased and then maintained a lower value as long as phenol was added. After the injection was stopped, however, their values increased sharply and then decreased again to the original control value (RBL $\approx$ 1) (Figure 2). Similar situations were seen with the TV1061 and DPD2794 channels. To reason for this is that, although toxic chemicals led to induced bioluminescences in these strains, the cellular metabolism would be depressed and cell death would occur when the chemicals were presents at concentrations over some critical value, thereby leading to a decrease in the RBL. Therefore, if the RBL values are lower than that of the control, the true response can be investigated further by diluting the samples. If the RBL value from the diluted sample is higher than that of the undiluted sample, this sample would then have a higher toxicity.

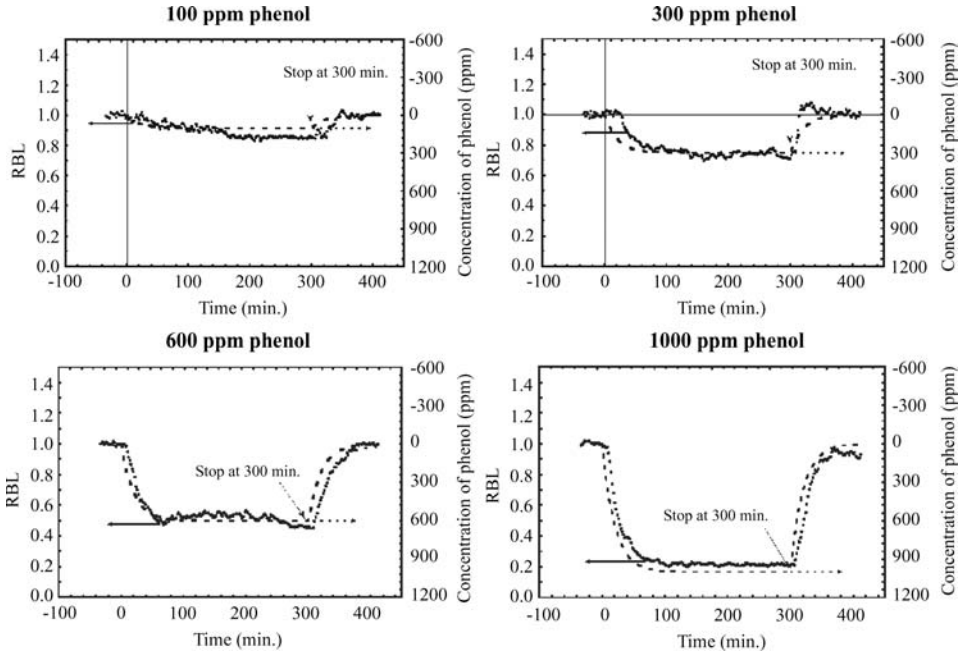

Figure 3. Responses of from GC2 to different phenol concentrations: (a) 100 ppm phenol, (b) 300 ppm phenol, (c) 600 ppm phenol, (d) 1000 ppm phenol. The dots are the measured bioluminescent values and the dashed lines are the concentration profiles in the 2nd reactor. A pulse-injection was used.

Due to the constitutive nature of the bacterial strain, GC2, this channel should show a decrease in its RBL proportional to increases in the phenol concentration. Figure 3 shows that this is the case. Because the GC2 strain emits bioluminescence constitutively, when toxic chemicals cause a metabolic burden or death, the bioluminescence decreases. After injection of the phenol sample, all concentrations tested showed an immediate response, and after reverting back to sterile water, the RBL values recovered to their initial level, i.e. the values before injection of the phenol solution. Therefore, this channel can detect materials that cause cellular toxicity leading to a metabolic burden or cell death.

Using a bell-shaped injection profile, it was possible to measure the responses of the strains to a steady increase and decrease in the toxic chemicals' concentration in the samples. For all the inducible strains, two RBL peaks were seen during the characterization. Below the critical concentration, the RBL continuously increased and then, as the concentration exceeded the critical value, it decreased. However, as the concentration decreased, the RBL value again increased. Finally, when the toxic chemicals were completely washed out of the second mini bioreactor, the RBL returned to the original control value. For the GC2 channel, however, the RBL values decreased in a manner similar to the concentration profile of the toxic chemicals (Figure 4). Evaluation and simulation results such as these can provide

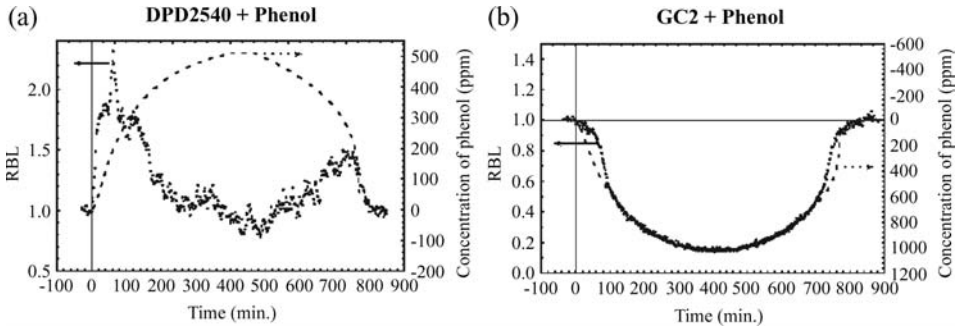

Figure 4. Responses of the bioluminescent strains (a) DPD2540 + Phenol and (b) GC2 + Phenol. The dots are the measured bioluminescent values and the dashed lines are the concentration profiles in the 2nd reactor. A pulse-injection was used.

standard signature responses and aid in the development of a database to indirectly determine a sample's toxicity. Although this system cannot determine exactly what toxic chemicals are present in the test samples, toxicity classification, which could not be performed by the classical fish or daphnia monitoring methods, is performed easily and efficiently.

3.2. TOXICITY TESTS WITH POWER PLANT DISCHARGES

The nuclear power plant sample resulted in various bioluminescent signals from the DPD2540 channel. At first, the undiluted sample water was injected into the 2nd mini-bioreactor of each channel. Although there was no significant response, when the injection was stopped the RBL of the DPD2540 channel increased suddenly as the sample was being washed out from the second mini-bioreactor. Therefore, a diluted sample (1:2 distilled water:discharge sample, volume ratio) was tested, and the RBL increased to a value of 1.9 (Figure 5(a)). Furthermore, when the sample was diluted in half its strength with water, the RBL increased to 1.5. The reason for the sudden increase in the RBL with the undiluted sample is thought to be that the original sample's toxicity is lethal. The results from the diluted samples support this since the dilution of the sample decreases the toxicity experienced to a level low enough to lead to a stable increase in the bioluminescence. As well, since the strain DPD2540 responds to membrane damage, it would appear that the water sample has some agent(s) that results in damage to the membranes of *E. coli* in solution.

For the thermo-electronic power plant discharge samples, the RBL values from the DPD2540 and TV1061 channels showed significant changes (Table I). The results for the TV1061 channel are shown in Figure 5 (b). In contrast to the nuclear power plant sample, though, dilution of the water sample led to a reduced response. However, the thermo-electronic plant used seawater as the coolant, and salt is known to result in membrane and heat shock due to osmotic stress to the bacterial

TABLE I
Maximum RBL values when the toxic chemical or field samples were injected into the second mini-bioreactor in a continuous manner

	Phenol (ppm)				MMC (ppb)				A power plant (Dilution ratio)			B power plant (Dilution ratio)	
Channel	100	300	600	1000	10	50	100	500	1	0.67	0.5	1	0.5
DPD2540	2.1	1.9	0.79	0.55	1.1	1.1	1.2	1.0	1.3	1.9	1.5	2.3	1.5
DPD2794	1.1	1.2	0.95	0.70	2.6	13.2	9.8	6.0	1.1	1.1	1.0	1.0	1.1
TV1061	1.5	2.3	1.7	0.84	1.2	1.1	1.2	1.2	1.1	0.98	1.1	3.2	2.3
GC2	0.85	0.71	0.46	0.19	1.0	1.0	1.2	1.1	0.94	0.96	1.1	1.0	0.97

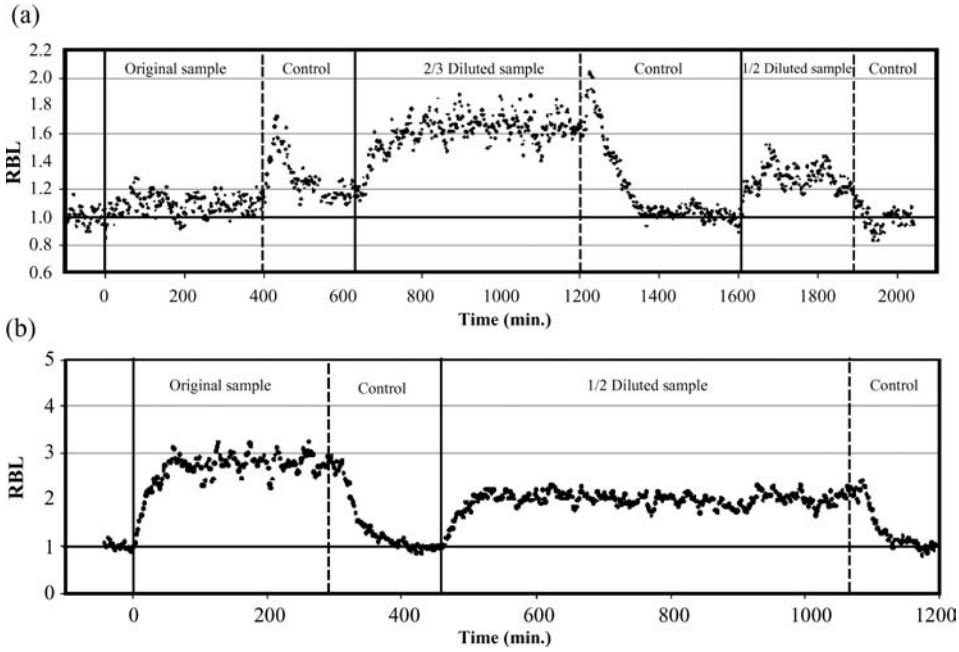

Figure 5. (a) Toxicity monitoring of the samples discharged from the 'A' nuclear power plant. The results shown are for the DPD2540 channel. (b) Toxicity monitoring of the samples discharged from 'B' thermo-electric power plant. The results shown are for the TV1061 channel.

cells (Allakhverdiev *et al.*, 2001; Xu *et al.*, 2001), and may be responsible for the responses seen in this study.

3.3. Comparison between the standard response and field samples

Specific bioluminescent levels from the simulation study can be used as standard tools to determine level of toxicity of field samples. In Table I, the maximum

RBL values are summarized for spiked samples and the samples from both power plants. Using a comparison between the RBL values of the standard tests and field tests, it is possible to estimate the toxicity of the field samples. Based on the significant responses seen, the nuclear power plant discharge sample appears to have some agent that causes membrane damage. Its toxicity level was similar with the responses seen when 100 to 300 ppm phenol was injected into the DPD2540 channel. In a similar manner, it is possible to estimate the toxicity of any field sample.

For the calibration of data generated from the constitutively bioluminescent expressed strains (GC2 channel), EC (effective concentration) or LD (lethal dose) values from fish or daphnia system for chemicals could be compared with bioluminescent inhibition directly. Relative toxicity could be obtained as depending on order of decrease of bioluminescent and EC or LD values among these systems (Kim *et al.*, 2003). The calibration of inducible strains (DPD2540, TV1061, and DPD2794 channels) was not fully established in this study. Information from these strains is distinct and unique as depending on target inhibition inside the cells. In this case, the change of bioluminescent levels at each channel, the shape of signals, and intensity of bioluminescent levels would be considered to evaluate the toxicity information of real field monitoring results for direct conversion or assimilation of simulated results to universal standard of toxicity.

Considering the complexity of real field samples from physicochemical conditions, the complete identification and classification of toxicity would be impossible using only the results generated here. The results, however, showed that the bioluminescent profiles are resembled with simulated data. These results indicated that it may be possible to predict the order of toxicity of field samples, depending on the bioluminescent levels and shapes generated from the simulated experiments. Although many standard signature datasets are needed for individual chemicals or their mixtures and calibration for toxic standard would be needed, this study demonstrates that this multi-channel continuous toxicity monitoring system and simulated data from the system is a novel tool for identifying the mode of toxic action. And this can be applied to many industrial outstreams for the monitoring of its effluents and discharges.

In the case of real field applications, some of issues also should be considered. The first one is the necessity of the sample treatment before loading into the reactors. Field water samples contain solid particles, which causes the prevention of flows as packing the tubing or cylinder connected to reactors and have unknown microbes, which lead contamination of pure cell cultured reactors as well as clogging in tubes or reactors due to cell aggregation. To overcome these, an appropriate filter system should be necessary for removing solid particles and microbes. In second, the values of bioluminescence measured by optical fibers could be transformed into PC based data acquisition system. As a result, web-based on-line monitoring and control of system would be possible with further development and modification in data acquisition and control system. Finally, the use of recombinant strains in the

fields is still quite limited and restricted, allowed in the laboratory for the purpose of research up to date. This system would be a first example to have a field test because the systems itself does not allow the recombinant strains to be released in the field.

4. Conclusions

1. Classification and detection of the toxicity within water samples were studied using the multi-channel continuous toxicity monitoring system in preparation for use with real field applications.
2. Standard signature datasets were obtained from simulation experiments using toxic chemicals to approximate the toxicity of field samples and closely mimic the conditions and concentration profiles of toxic chemicals' present in real situations.
3. This novel continuous toxicity monitoring system reduces the time needed for an early-warning and offers an alternative for the monitoring and control of water quality, as well as aids in set up of new monitoring strategies to protect the sources of tap water and to prevent polluted water from being discharged from industrial sources.

In conclusion, this system estimates the toxicity of field samples based on a standard signature data set and, therefore, can be applied to industrial or waster water treatment plants as an instrument to maintain water quality control.

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