Increased toxicity of textile effluents by a chlorination process using sodium hypochlorite

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Abstract Chlorinated textile effluents were tested for their toxicity using different bioassays. These assays were the Microtox® assay, daphnia (*Daphnia similis*) 48-hr survival test, medaka embryo 14-day and juvenile 96-hr survival tests, and tilapia (*Oreochromis mossambicus*) juvenile 96-hr survival test. By comparing the results of toxicity tests on water samples collected at the instream prior to the chlorination process and at the outlet of the wastewater treatment facility, we found that wastewater toxicity was obviously increased by chlorination using NaOCl as the oxidant, as evidenced by the different bioassays used. Because no significant difference was observed in water chemistry, such as pH, DO, and conductivity, the induced-toxicity may be partially attributable to residue chlorine or other chlorinated compounds generated by chlorination. Future studies are warranted to identify the cause of the increase in the textile wastewater toxicity.
Keywords Textile effluent; Microtox®; *Daphnia similis*; Japanese medaka; tilapia; bioassay

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Introduction

In many developed countries, toxicity tests on an industrial effluent are required to ensure that the discharge will not have adverse effects on the aquatic organisms in the receiving water. For instance, the US EPA has incorporated different aquatic toxicity tests in National Pollutant Discharge Elimination System (NPDES) permits since 1984. In Taiwan, however, local government still relies on chemical or physical characteristics of water quality to monitor and regulate a point source discharge. In an initial study on industrial effluent toxicity, we have found that a textile effluent showed moderate toxicity to some, but not all, aquatic species. After that investigation of the textile effluent, in order to comply with the Taiwan EPA's new COD (chemical oxygen demand) standard of 100 mg/L and the color standard of 400 American Dye Manufacturer Institute's (ADMI) units, the same textile wastewater treatment plant added a chlorination process before the effluent outlet using sodium hypochlorite (NaOCl). However, it was known that the chlorination could increase effluent toxicity because of the high level of residual chlorine in water. Furthermore, formation of more toxic chlorinated or oxidized organic compounds in the wastewater could occur, leading to a change in effluent toxicity (Dinnel and Stober, 1987). The aim of study was to determine if chlorination of the textile wastewater will result in alteration of textile effluent toxicity. Different aquatic toxicity tests were employed in this study, namely the Microtox® assay, daphnia (*Daphnia similis*) 48-hr survival test, medaka embryo 14-day and juvenile 96-hr survival tests, and tilapia (*Oreochromis mossambicus*) juvenile 96-hr survival test.

Methods

The textile plant located in Quan-Tein, Tainan County, Taiwan. The chlorination unit was installed onto a post-coagulation tank right after the secondary treatment (Figure 1). Prior to the installation, characteristics of the effluent were: flow rate= 5000 m^3/day, suspended

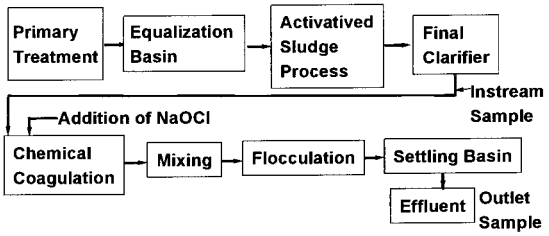

Figure 1 Wastewater treatment process in the investigated textile plant

solids (SS)= 18~25 mg/L, pH= 6.4~6.8, COD= 150~200 mg/L, color= 800~1000 ADMI units. Some of these parameters have changed to pH=6.4~7.0, COD<100 mg/L, color<200 ADMI units afterward. This study was comprised of two stages depending on the timing of sample collections. At the first stage in which chlorination unit has not yet been installed, wastewater samples were taken from the effluent outlet. At the second stage, after the completion of chlorination unit installation, water samples were collected from two locations. One was at the instream of the final chemical coagulation tank at which NaOCl, with a dosage of 300~400 mg/L, was added, and the other was at the outlet of the wastewater treatment facility (Figure 1). Upon collection, water samples were immediately transferred to our laboratory and refrigerated at 4°C for future analysis. ADMI Tristimulus Filter Method (Method 2120E in *Standard Methods*, 1995) was used to determine the color of samples. COD was analyzed based on the procedure described in method 5220C in *Standard Methods* (1995). Residual chlorine was measured by an Aquamerck® Chlorine kit based on the colorimetric method. The sensitivity range is between 0.1 and 2 mg/L.

Each water sample was tested for toxicity twice (repeated test) two to three weeks apart (due to space limitation and animal availability in our laboratory, they could not be run simultaneously). Water samples from each location were taken 3 times at different periods, and a total of 9 samples were obtained and tested for their toxicity using different aquatic species.

Fish species used in this study included the Japanese medaka (*Oryzias latipes*) and tilapia (*Oreochromis mosssambicus*). The Japanese medaka used to be widely spread in fresh water in Taiwan, but now they could only be found in pristine water due to degradation of water quality throughout the island. Euryhaline tilapia is now the dominant fish species in rivers and lakes in Taiwan because of its tolerance to environmental deterioration in recent years. The other two aquatic species used in this study were daphnia (*Daphnia similis*) and luminescence bacteria (*Photobacterium phosphoreum*), also known as the Microtox® assay. Cadmium chloride ($CdCl_2$) and sodium dodecyl sulfate (SDS) were used as reference toxicants in all assays, except in the Microtox® assay, in which phenol was used as proposed in the guideline (Microbics Corporation, 1992).

The medaka assay was comprised of two parts. One was the 96-hr static acute toxicity test using juvenile fish, and the other used its embryos to examine the developmental effects of wastewater. The toxic endpoints in the latter were embryo hatchability, morphological abnormality and survival rate after an approximately two-week incubation.

A ninety-six hour acute toxicity test was adopted from the guidelines for *Pseudorasbora parva* (Taiwan EPA, 1994, NIEA B902.10T) with a minor modification. Briefly, medaka juveniles were transferred to 3 L glass containers containing different concentrations of a water sample, and three concentrations of $CdCl_2$ or SDS for positive control at 22~26°C for 96 hours with a photoperiod of 16:8. Each concentration had two batches (replicate), and each batch contained 10 fish. The number of dead fish was recorded at 2, 6, 24, 48, 72, and 96 hours, and each carcass was removed immediately. In each experiment, water characteristics, such as pH, temperature, dissolved oxygen (DO), and conductivity were monitored

daily. LC50s (median lethal concentrations) were calculated by the ToxCalc^{TM}5.0 (Tidepool Scientific Software), and were expressed as the percent dilution of the wastewater. The computer software employed maximum likelihood regression or Spearman-Karber method in estimation of LC50 values, depending on variability of the data. It should be kept in mind that the smaller the LC50 or EC50 value, the more toxic a water sample. The same setup and process were applied to tilapia juvenile assay as well. And the assays on two fish species were conducted simultaneously.

The medaka embryo assay was adopted from Wisk and Cooper (1990). Medaka eggs were collected from female abdomen by gently scratching with a fine mesh net. After reaching the 64-cell stage of development, each egg was placed into a 2 ml Teflon® capped glass vial containing either different concentrations of a water sample or one of four concentrations of $CdCl_2$ or SDS. Various concentrations of a water sample were prepared by different dilutions with a rearing solution which was prepared according to Kirchen and West (1976). Embryos were observed daily with a dissecting microscope for their development, and were recorded for any abnormality until they hatched at about 10~14 days incubated at 27±1°C. The LC50s for embryo mortality and EC50s (median effective concentrations) for hatching success were calculated using the ToxCalc^{TM}5.0 software.

Daphnia are frequently used in aquatic toxicity testing. *Daphnia similis* is an indigenous Cladocera species to Taiwan. Taiwan EPA also published a guideline for conduction of acute toxicity tests using this species (Taiwan EPA, 1997, NIEA B901.11B). The same guideline was used in this study. Briefly, daphnia neonates, less than 24 hours of age, were transferred from rearing medium into 100 ml beakers containing various concentrations of the wastewater with a volume of 50 ml. Animals were incubated at 25 ±1°C and a photoperiod of 16:8 hr in an incubation chamber during the testing period. Each concentration had four replicates (beakers) and each beaker contained five animals. Test water's pH, temperature, DO, and conductivity, as well as the daphnia's mortality (immobility) were measured and recorded daily. After 48 hours, EC50s (daphnia immobility) were calculated using the ToxCalc^{TM}5.0 software. SDS and $CdCl_2$ were used as the reference toxicants in this assay.

The Microtox® assay was also used in this study to determine the acute toxicity of the textile wastewater. A Model 500 Microtox® unit and a standard procedure proposed by Microbics Corporation (1992) were used in this assay. Wastewater samples were serially diluted and mixed with a reagent containing activated bacteria. Fluorescence emission was measured at the 5 and 15 minutes after incubation of the mixed solution at 15°C. Both 5-min and 15-min EC50s (50% inhibition of luminescence) of water samples were determined, but only 15-min EC50 values were reported.

Results and discussion

In this study, we used different aquatic organisms to test the toxicity of a chlorinated textile effluent. The results of this study were shown in Figure 2. At the first stage (before installation of a chlorination unit), the toxicity of the textile effluents from the same wastewater treatment plan varied with time and toward different assays used. For instance, in the medaka 96-hr assay, the first two effluent samples, which were designated as Collection A and B (Figure 2A and B), showed no toxicity, while the third sample (Collection C), tested twice at two different time periods, had LC50s of 66.5% and 95.3%, respectively. But in the medaka embryo and daphnia assays, all three samples showed hatching inhibition on the medaka embryo and immobilization effects on the daphnia. The Microtox® assay was not conducted in the first stage study.

By comparing the results from toxicity tests on water samples collected in the first and second stages, we found that they were very similar with respect to susceptibility of different bioassays, as well as temporal variation in toxicity. In general, both water samples showed

no or moderate lethal toxicity to juvenile fish and medaka embryos, while medaka embryo hatching and daphnia immobility were more sensitive than the other toxic endpoints.

In the second stage study, an instream water sample from the first collection (Collection A) showed neither acute toxicity to tilapia and medaka juveniles, nor to *Daphnia similis.* But it had effects on medaka embryo's survival and hatching success, with the LC50 and EC50 of 42.3% and 48.5%, respectively. It also inhibited photobacterium luminance in the Microtox® assay with a 15-min EC50 of 33.6%. However, in the repeated test on the same sample, the wastewater became acutely toxic to tilapia juveniles with a LC50 of 90.5%.

In contrast, bioassays on the water sample of Collection A collected at the outlet of the wastewater treatment facility showed that it was toxic to all aquatic species used with different sensitivities between assays (Figure 2A). It should be noted that this sample was collected at the same time as the sample of instream Collection A. The most significant observation was that the LC50s and EC50s from different assays were all decreased

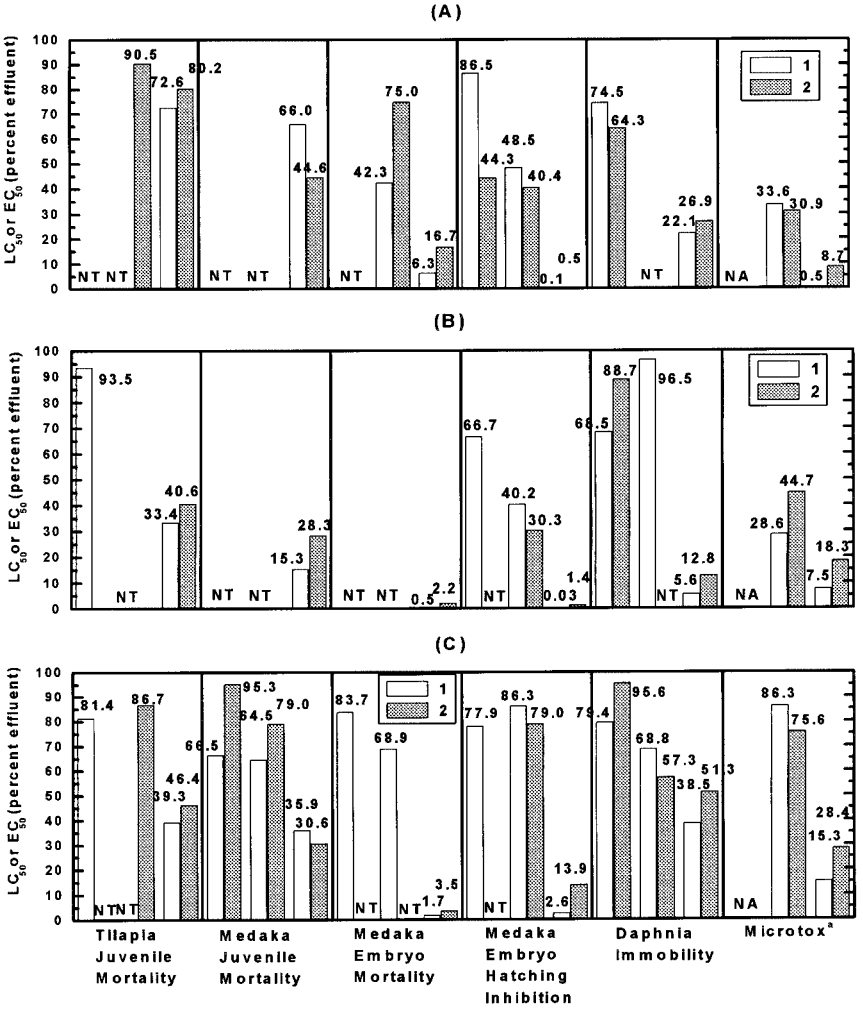

Figure 2 EC50s and LC50s (percent effluent) of the three collection wastewater samples (A, B, and C) tested by different bioassays. Bars with two different patterns (1 and 2) represent two tests on a sample at different times. Three groups of bars in an assay represent three different water samples collected at different locations or time periods. The first group was the effluent samples collected prior to the installation of the chlorination unit (first stage of this study). The second was the instream sample and the third was the outlet sample collected in the second stage. NT indicates that the water sample was not toxic. NA indicates that the test was not conducted, and data were not available. The number above a bar corresponds to its actual value.

compared to the results from tests on the instream sample, indicating an increase of the wastewater toxicity (Figure 2A). For example, in the medaka embryo assay, EC50s for hatching inhibition of 48.5% and 40.4% from the two tests (one was the repeated test) on the instream sample were much higher than the EC50s of 0.1% and 0.5% from the two tests on the outlet sample. The induced toxicity was about 500 fold. Moreover, instream water sample was not acutely toxic to daphnia (no EC50 value in Figure 2A), but the outlet sample was toxic with EC50s of 22.1% and 26.9% from two tests. The result of the Microtox® assay also showed a higher toxicity of the outlet water sample than the instream one. Other assays, including juvenile 96-hr and medaka embryo survival tests, all indicated that there were differences in toxicity between two water samples (Figure 2A). Although these differences between LC50s or EC50s look significant, they cannot be validated with statistical analysis due to relatively small sample size.

Results of different toxicity tests on the second collection wastewater sample (Figure 2B) suggested a trend similar to that of the Collection A, in which water samples obtained from the outlet were more toxic or became toxic to testing organisms than samples from the instream. The instream sample from the second collection (Collection B) was only toxic to medaka embryos (in inhibition of hatching), daphnias, and photobacteria in the Microtox® assay (Figure 2B). But tests on the outlet sample showed that the toxicity was increased to some extent. It not only caused mortality of tilapia and medaka juveniles, and medaka embryos (LC50: 33.4% and 40.6%, 15.3% and 28.3%, 0.5 and 2.2% in the two tests), but also decreased the EC50 values of embryo hatching inhibition up to 50 fold, from 40.2% and 30.3% (average 35.3%) to 0.03% and 1.4% (average 0.7%) (Figure 2B).

Toxicity tests on the water sample from the third instream collection (Collection C) showed varied results comparing to those from the first two collections. It was not toxic to tilapia juveniles at the first test. But in the repeated test, the same sample showed a LC50 of 86.7%. In contrast, the outlet sample resulted in lower LC50 values (more toxic), which were 39.3% and 46.4% of the two tests. In the medaka juvenile assay, instream samples from the first two collections were both toxic to medaka, but the instream water sample from the third collection had LC50s of 64.5% and 79.0% (average 71.8%) in the two tests. The outlet sample, however, had much lower LC50s values, 35.9% and 30.6% (average 33.3%) of the two tests. The increase of toxicity was about two fold (Figure 2C).

Using medaka embryos assay, we also observed an increase in toxicity comparing instream to outlet water samples from Collection C. The first test on the instream sample showed a LC50 of 68.9% in embryo mortality, while the second test was not toxic (Figure 2C). When the outlet sample was examined, it showed a significant increase in embryo toxicity. The LC50 values calculated from the two tests on the outlet water sample were down to 1.7% and 3.5% (average 2.6%), respectively. The difference was greater than 25 times (68.9% vs. 2.6%). In contrast, the average EC50 of hatching inhibition for the instream sample from Collection C was 82.7% (86.3% and 79.0% in the two tests), but the average EC50 for the outlet sample was only 8.3% (2.6 and 13.9% in the two tests).

In daphnia and the Microtox® assays, the toxicity of the outlet sample of Collection C was also greater than the instream one. Survival (mobility) of *Daphnia similis* was affected by the instream water with an average EC50 of 63.1% (68.8% and 57.3% in the two tests), while outlet water resulted in an average EC50 of 44.9% (38.5% and 51.3% in the two tests). The difference in Microtox® toxicity between instream and outlet samples from the third collection was more obvious (Figure 2C). The EC50 value of the outlet sample (average 81.0%) was about four times higher than that of the instream sample (average 21.9%).

The results of this study strongly indicated that the toxicity of the textile wastewater was elevated by the chlorination process. Despite the various sensitivities of the different assays and temporal variation in toxicity responses, the LC50 and EC50 values from the tests on

outlet samples were all less than those from tests on instream samples. These results were similar to many studies on paper/pulp mill effluents, in which chlorine or chlorine-based chemicals were used in the bleaching process. For instance, using the Microtox® assay, Kim Oanh (1996) found that the hypochlorite bleaching stage effluents from two of the three studied pulp and paper mills were the main contributors to acute toxicity of the discharges. There were only a few studies on the induction of toxicity due to chlorination processes in other industrial effluents. In an investigation of alternatives to chlorination for disinfection of wastewater, Venosa and Ward (1978) found that a wastewater became toxic to fathead minnows (*Pimephales promelas*) after chlorination, but was detoxicated after a dechlorination process. In a study on the effect of treated municipal wastewater on sea urchin fertility, the toxicity was found to be restored by chlorination (Dinnel and Stober, 1987). Blatchley III and his colleagues (1997) have reported the effects of different disinfectants, including chlorine, on toxicity of different wastewater effluents, assessed by survival and reproduction of *Ceriodaphnia dubia*. In their study, they summarized that the disinfection process only altered the toxicity of an effluent that was toxic beforehand. However, they also cautiously concluded that disinfectant-induced toxicity could not be assumed to occur at all wastewater treatment facilities.

The induced toxicity of effluents observed in this study may result from the changes in water characteristics. We have measured some chemical parameters of each water sample and monitored throughout each test. There were no significant changes in different parameters, such as pH, temperature, dissolved oxygen, COD, and conductivity, between instream and outlet samples or between water samples used in two repeated tests. The residual chlorine concentration varied and ranged from 0.1~2 mg/L. This level should be considered toxic to the aquatic species used in this study, because there have been reports indicating that LC50 values of the total residual chlorine for different aquatic species were in the ten to several hundreds ppb (μg/L) range. For instance, the acute 96-hr LC50s for continuous chlorine exposure of the mysid (*Mysidopsis bahia*) and the inland silverside (*Menidia beryllina*) were 73 μg/L and 128 μg/L, respectively (Fisher *et al.*, 1994). Morgan and Prince (1977) determined 24- and 48-hr LC50s of total residual chlorine to be 380 and 300 μg/L for embryos of Atlantic silverside (*M. menidia*). It should be mentioned that the residual chlorine concentration in water declines with time. So, when the water samples were taken out from storage for toxicity tests, their residual chlorine concentrations could decline tremendously. Although we did not measure the residue chlorine levels in the test solution throughout each test, we did analyze them before testing. Their concentrations were significantly decreased after a 2-day storage (down to less than 100 μg/L). This may explain why the LC50 or EC50 values from the repeated test (the second test) on a sample were higher (less toxic), in most of the cases, than those from the first test on the same water sample, despite the different assays used (Figure 2). However, it was also noted that after a 2~3-week storage, water samples still exhibited toxicity. This suggested that at least some toxic constituents present in the textile wastewater after chlorination might not dissipate in such a time period. This also indicated that residual chlorine alone should not be responsible for all the wastewater toxicity induced by chlorination. Szal and his colleagues (1991) have correlated the residual chlorine concentrations to effluent toxicity to fathead minnow survival. However, in that study, the toxicity of total residual chlorine in the field study varied substantially among sites. For example, at one site, a total residual chlorine concentration of 0.1 mg/l resulted in 100% mortality to minnows, while at another site, 80% or more of the minnows tolerated the total residual chlorine levels up to 0.35 mg/L.

In addition to residue chlorine, which may diminish with time in the water, volatile organic compounds (VOCs) also exhibited a similar property. It is known that some chlorinated VOCs, such as trihalomethanes, could be generated by chlorination of water. A study

on disinfection byproduct formation due to chlorination of secondary effluents indicated that trihalomethanes and some volatile haloacetic acids were formed in high concentration during chlorination (Rubhun, 1997). Therefore, if these VOC contributed some toxicity of the textile effluent to aquatic organisms used in this study, the decrease in concentrations due to volatilization could also result in reduction of toxicity observed in the second test (repeated test) of the same water sample.

Traditional textile wastewater is complex in nature, and contains a variety of chemicals. These include waxes, oils, dyes, surfactants, fixatives, chelating agents, inorganic salts, dye carriers, softeners, formaldehyde-based resins, latex products, etc. The toxicity induced by chlorination may be partly due to formation of other toxic organic substances not previously present. Of course, questions exist on whether these chlorinated or oxidized substances are present in concentrations high enough to cause adverse effects on organisms tested. Moreover, traditional textile wastewater not only contains a variety of organic compounds, but also metals. Using the Toxicity Identification Evaluation (TIE) protocol, Wells *et al.* (1994) concluded that a textile wastewater effluent exhibited a low degree of toxicity to *Daphnia pulex* in acute, static, 48-hr tests, and zinc was the major contributor to the observed toxicity. But according to our information, no metal was involved in any of the process in the textile facility from which our water samples were collected. Therefore, metals should not be responsible for the toxicity observed. Besides, if metals in the wastewater were present in the amount capable of eliciting any adverse effect on the test organisms, they would have demonstrated the toxicity in the tests on instream water samples. Finally, it is worth mentioning that the magnitudes of the increases in toxicity observed in different assays were varied, some significantly. This suggests that different species responded differently to the changes in wastewater characteristics attributable to the chlorination process.

To summarize the results of this study, the textile wastewater prior to chlorination showed moderate toxicity in some of the bioassays, namely the medaka embryo hatching inhibition test, the *Daphnia similis* immobility test and luminance inhibition in the Microtox® assay. But after the wastewater was chlorinated with sodium hypochlorite (NaOCl), it either became toxic or was more toxic to different organisms or different toxic endpoints tested. The increases in toxicity due to the chlorination process were obvious, but they did not result from differences in water chemistry, such as changes in pH, DO, and conductivity. Residual chlorine may partly contribute to the toxic effects observed in this study, but other chlorinated or oxidized organic chemical, e.g. trihalomethanes, could also be responsible. It is possible that the toxicity induced by chlorination may not limit to one, but a combination of several factors (constituents) in the textile wastewater.

Due to complexity of the textile effluent chemistry and the dynamics of the chlorination process, either the mechanism(s) or agent(s) related to the increased toxicity were uncertain. However, in this case, if the Toxicity Identification Evaluation protocol could be initiated, the nature of the causative agents would then be defined. And this, in turn, could serve as the basic and essential information for the Toxicity Reduction Evaluation (TRE) process in the future. Nevertheless, the results of this study emphasize that instead of using NaOCl or other chlorine-based chemicals, alternatives for color removal or COD reduction from the textile effluent should be considered because of either their toxicity in themselves or potential for alteration of whole effluent toxicity (WET). Intermittent chlorine exposure was less toxic to mysids and inland silversides in acute and short-term chronic tests (Fisher *et al.*, 1994). Liao *et al.* (1999) has evaluated the possibility and the cost of H_2O_2/UV oxidation treatment of the same textile wastewater as in this study. In that report, they concluded that H_2O_2/UV oxidation was highly cost-effective in COD reduction and color removal. In an investigation of effects of different disinfectants on wastewater effluent toxicity, Blatchley III (1997) found that the changes in wastewater toxicity attributable

to disinfectant exposure followed the trend: chlorination/dechlorination> ozonation> UV irradiation. This information could be used as an important reference or a guide for related practices. Nevertheless, evaluation and caution must be taken before employing any treatment operation in view of alteration of whole effluent toxicity.

Conclusions

Textile wastewater was tested for toxicity using different bioassays in this study. By comparing test results on instream (before chlorination) and effluent water samples, we found that the wastewater toxicity was increased significantly by chlorination, despite various assays employed. With respect to water pollution prevention and protection, integration of bioassays, other than a traditional approach, into environmental monitoring of effluents should be essential. Appreciation of this principle is especially important to countries like Taiwan, in which regulation of industrial effluents is still based solely on chemical and physical properties of an effluent without consideration of their biological (toxicological) characteristics. Furthermore, using multiple-species bioassays could ensure that such diverse characteristics in wastewater effluent could be revealed as possible. And this will provide essential information for sound environmental/ecological management on the aquatic environment.

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