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Review

Bioassay based luminescent bacteria: Interferences, improvements, and applications

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HIGHLIGHTS

- This paper shows the interferences and improvements of luminescent bacteria test.
- This review indicates the future research direction for luminescent bacteria test.
- The luminescent bacteria test is moving toward high automation and reproducibility.
- Review shows the factors that affect the toxicity of chemicals and actual waters.
- A battery of bioassays and chemical analysis are recommended for sample evaluation.

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ABSTRACT

Due to the merits of being time-saving, cost effective and simple operation, the luminescent bacteria toxicity assay (LBTA) has been widely used for environmental pollution monitoring. Based on numerous studies since 2007, this critical review aims to give an overview on the mechanisms, developments and applications of LBTA. Firstly, based on the introduction of the mechanisms of LBTA, this review shows the interferences from the characteristics of testing samples (such as inorganic nutrients, color, turbidity) and summarizes the improvements on pretreatment method, test methods and test systems in recent years. Regarding the factors that affect the toxicity prediction of single chemicals, the correlation between the toxicity index expressed as median effective concentration (EC_{50}) and characters (such as Kow, the alkyl chain length, the anion and the cation) of known chemicals, especially the emerging ionic liquids (ILs), were given an in-depth discussion. The models for predicting the joint effect of mixtures to luminescent bacteria were also presented. For the factors that affect the toxicity of actual waters, the correlation of toxicity of actual samples to luminescent bacteria and their conventional indexes were discussed. Comparing the sensitivity of the LBTA with other bioassays could indicate the feasibility of the LBTA applied on specific samples. The summary on the application of LBTA to environmental samples has been made to find the future research direction.

Contents

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1. Introduction

Increased attention to the impact of toxic substances on aquatic ecosystem and human beings, leads to the development of sensitive and observable organisms as bio-indicator for the environmental pollution monitoring and management. In comparison with the chemical analysis that is only able to determine the components and concentrations of the contaminants released in the environment, biological analysis could intuitively reflect the detrimental effects of contaminants on organisms and profoundly reveal the mechanisms of the toxic effects including formation, development and removal (Li et al., 2013a, 2013b). However, biological analysis tends to be time consuming, and it requires professionals to cultivate the organisms. Due to the potential impact of contaminants, especially new synthesized chemicals in aquatic ecosystems, the need for a fast, convenient, and cost-effective method to assess biological toxicity is becoming very necessary. The application of luminescent bacteria on toxicity assay captured the researchers' attention and was quickly developed.

Luminescent bacteria can emit blue-green light in the process of the normal metabolism. The first luminescent bacterium strain was named by Heller J.F. in 1854, and early reference for assessing the acute toxicity dated to 1930s, t. The LBTA of water samples was commercialized as Microtox® test by Beckman Instruments, Inc. U.S.A. in 1978 (Flokstra et al., 2008). After that, many countries and regions have established the official standard for luminescent bacteria inhibition assay applied on water samples, such as French standard (DIN 38412-1990), American standard (ASTM D5660-1995), Chinese standard (GB/T 15441-1995) and European standard (EN ISO 11348). Presently, many countries have set toxicity limits to luminescent bacteria for effluent discharge (Rodrigues and Umbuzeiro, 2011).

Parvez et al. (2006) have reviewed the test procedure, the variations and the advantages of bioluminescence inhibition assay comparing with other acute toxicity test. Based on researches published during the years 2000–2007, Girotti et al. (2008) gave an overview of the applications of luminescent bacteria on toxicity assay, including analysis by natural luminescent bacteria, cellular components, recombinant bacteria, and biosensors etc. Nevertheless, the bioassay using luminescent bacteria has been further developed and the versatility of LBTA has been increased based on plenty of researches in recent years.

For providing more overview aspects of LBTA, this review seeks to discuss the interferences and improvements of LBTA, to analyze the factors that could affect the toxicity of known chemicals and actual water samples, to compare the LBTA with other bioassays, and to summarize the application of LBTA on environmental monitoring. Future perspectives and research needs of LBTA are also identified based on this extensive review. This review only represents the toxicity assay using whole-cell luminescent bacteria. The toxicity analysis with cellular components are not within the scope of this review (e.g. the methods to determine bacteria luciferase and the NAD(P)H:FMN oxidoreductase).

2. The mechanism of luminescent bacteria assay

2.1. The luminescent bacteria

Luminescent bacteria are ubiquitously distributed in nature, e.g. seawater, sediment, skin of fishes. The major part of luminescent bacteria is marine species except the freshwater *Vibrio qinghaiensis sp.Q67*, the freshwater *Vibrio cholerae* and the terrestrial *Photorhabdus* species. The light emitted by luminescent bacteria principally relies on the bioluminescent enzyme system which consists of a NAD(P)H:FMN oxidoreductase and a luciferase. Firstly, FMN reduces to $FMNH_2$ upon the reaction catalyzed by NAD(P)H:FMN oxidoreductase enzyme in the presence of a reduced NAD(P)H and a H^+ (Eq. (1)).

$$NAD(P)H + FMN + H^+ \xrightarrow{\text{NAD(P)H:FMN oxidoreductase}} NAD(P)^+ + FMNH_2 \quad (1)$$

Then bacterial luciferase catalyzes the oxidation of reduced $FMNH_2$ to produce oxidized FMN in the presence of long chain aldehyde (RCHO) and O_2 with the light emission (Eq. (2)) (Inouye, 1994). The spectral range of the bioluminescence is between 420 nm and 660 nm. The bacterial luciferase that plays an important role in the bioluminescence reaction is a heterodimeric protein (Matheson and Lee, 1981). The genes (named *lux* gene) for luciferase production are present in every luminescent bacterium as a coordinately expressed set of genes, *luxCDABEG*, which is the *lux* operon (Meighen, 1991). Fig. 1 shows the *lux* genes of *A. fischeri* (named *Vibrio fischeri* before), *Vibrio harveyi* and *P. phospherium* which are often employed in toxicity assay (Urbanczyk et al., 2007). To date, the *lux* operon of luminescent bacteria has been often used to create recombinant stains for bioassay. Taking *Anabaena* CPB4337 for example, it is based on *Anabaena* sp. PCC 7120 strain CPB4337 bearing in the chromosome a Tn5 derivative with *luxCDABE* from the terrestrial *P. luminescens* (Rodea-Palomares et al., 2010; Rosal et al., 2010).

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + h\nu \quad (2)$$

2.2. The luminescent bacteria assay

The light emission is closely related to cellular metabolism, and thus light intensity reflects the metabolic status of the bacteria. When the luminescent bacteria are exposed to toxic substances, the bacterial luciferase could be inhibited and the light intensity decreases rapidly. By measuring the light intensity of the bacteria exposed to testing sample and comparing with that of control, the inhibition can be calculated for quantifying the toxicity to luminescent bacteria. That is the principle of traditional luminescent bacteria inhibition assay which has been employed as the standard of many countries and regions. The most widely used index for bioassay is EC_{50} which is the concentration of toxicant corresponding to the inhibition value of 50% at a certain exposure time. For the convenience of comparison, the toxicity units (TU) and

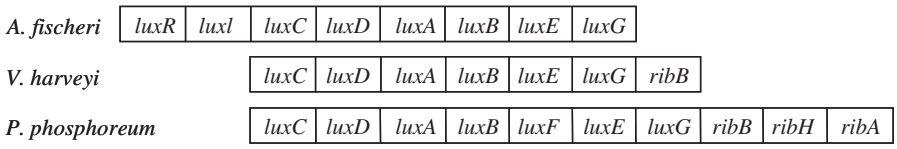

Fig. 1. The *lux* genes of luminous bacteria (adapted from Dunlap, 2009).

toxicity impact index (TII_{50}) defined as the reciprocal of EC_{50} are also used to express the toxicity.

Concerning the toxicity assay based on marine luminescent bacterium, such as the most widely used stain *A. fischeri*, the testing samples need to be prepared in 2%–3% NaCl to mimic an environment of sea water. However, this may change the characters of the testing sample, such as decreasing the metal bioavailability and increasing the insolubility of organic substances (Gao et al., 2012). As an alternative, *V. qinghaiensis sp.Q67* is a freshwater luminescent bacterium that does not need to maintain a salt environment and has a similar mechanism of light emission (Ye et al., 2011). The *V. qinghaiensis sp.Q67* inhibition assay may be a promising method for toxicity evaluation even though there are not many commercial test kits for *V. qinghaiensis sp.Q67*, contrasting with *A. fischeri*.

The LBTA is often recognized as the acute toxicity on account of the short exposure time. In Microtox® test, the exposure time is 5 min or 15 min. Actually, the luminescent bacteria is not only available for measuring the short-term/acute (5–30 min) toxicity based on the light inhibition due to a disturbance on the photosensitization activity and but also long-term/chronic (12–24 h) toxicity based on the changes in viability or growth rate of the bacteria. In addition, the long-term toxicity bioassay allows a dynamic analysis of toxicity to proceed with the data collected over a certain period of time in the whole exposure time.

3. Both the interferences and improvements of LBTA

3.1. The interference emerging in LBTA

The variations of the traditional LBTA caused by the test procedure or the testing materials have been clearly declared in the established standards. In these years, the researches aimed to modify the traditional LBTA method for increasing the sample throughput and improving the sensitivity. The modified methods usually accompany the new variations which need to be paid more attention to. Recently, luminescent bacteria inhibition assay has been miniaturized with the new microplates toxicity analysis (MTA) for decreasing the costs and obtaining the high throughput. The MTA are often performed with white or black plastic 96-well microplates while the basic tests are conducted with glass cuvettes or tube. Generally, the white microplates are recommended for luminescence determination due to their light enhancing property. Yet, for the colored testing sample, the white microplates could multiply absorb the light reflected by the walls of the wells, which lead to overestimate the toxicity of samples (Hirmann et al., 2007). Hence, the application of black microplates on MTA is recommended for colored sample.

An interesting phenomenon in toxicity assay based luminescent bacteria is the hormesis effect of toxic compounds and the stimulating effect of actual samples, which are the light intensity of the bacteria exposed to testing sample larger than that exposed to control. The toxic compounds usually have hormesis effect to organisms at low concentration whilst the actual samples containing abundant nontoxic nutrients also have stimulating effect to luminescent bacteria.

The stimulating effect of inorganic nutrients (e.g. K^+, Ca^{2+}, Na^+) with low concentrations has been observed on natural luminescent bacteria before. That could increase the variation of luminescent bacteria toxicity test and even indicate false results. The stimulating effect was not relieved using a freshwater recombinant strain (Deriabin and Aleshina, 2008). A suitable control sample might weaken the stimulating effect. The standard diluent for marine luminescent bacteria is 2% NaCl solution, which was used for preparing the solutions of chemicals and control samples. Considering the stimulating effect of inorganic nutrients, the diluent consisting of NaCl, KCl and $CaCl_2$ may be used in toxicity assay for maintaining the bacteria with maximum light production. Unfortunately, the toxicity of contaminants to luminescent bacteria was underestimated using this optimized diluent (Berglind et al., 2010). On the other hand, low concentrations of PO_4^{3-} and CO_3^{2-} increased the toxicity of heavy metal (Hg^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}) to a freshwater recombinant strain, indicating their modulating effect on the bacterial uptake of heavy metals (Rodea-Palomares et al., 2009). However, the *A. fischeri* toxicity assay using biotic ligand-based models is a promising method for predicting the toxicity of single heavy metals in testing samples and decreasing the interference from the cations (Ca^{2+}, Mg^{2+}, K^+) (Luo et al., 2008; An et al., 2012).

In 1990, Microtox® solid phase assay (MSPA) was developed for solid sample detection and was established in detail in 2005, expanding the application range of LBTA (Burga Pérez et al., 2012). With respect to the protocol of MSPA, *A. fischeri* contact with a series of sediment dilutions for 20 min, then the filtrates containing the luminescent bacteria are obtained by separating the liquid from the sediment using the disposal column filters. After a certain exposure time (5 min, 10 min, 15 min or 30 min), the filtrates are put into the instrument for the measurement of luminescence. Similarly with water sample, the MSPA could be affected by the characters of the filtrates obtained from the sediments, such as inorganic chemicals, pH, color and turbidity (Volpi Ghirardini et al., 2009; Karlsson et al., 2010; An et al., 2012). The soil and sediments often contain amounts of fulvic and humic acids and heavy metals. The fulvic and humic acids maybe also have a potential effect on the interference of MSPA as it has been proven that they could bind with various metals and decrease their bioavailability (Koukal et al., 2007). Nevertheless, the important impact factor is the initial light loss caused by an adherence of bacteria to particles in sediments in the exposure time and the removal of bacteria in the process of filtration, indicating an overestimated result (Mamindy-Pajany et al., 2012). For decreasing the initial light loss in MSPA, Burga Pérez et al. (2012) proposed an effective method that is combining flow cytometry analyses with MSPA to make a correlation of EC_{50} value. However, the Microtox® leachate phase assay (MLPA) that they mentioned may not be an available method to avoid the initial light loss. Through their further study, it was found that bacteria also could fix to the fines in the filtrates and the fines could bind the contaminants and decrease their bioavailability (Perez et al., 2013).

3.2. The improvements of LBTA

A useful pretreatment method could not only eliminate the interferences but also obtain the target substances from the testing sample. Some special pretreatment methods applied on other bioassays are also available for LBTA. The LBTA only needs small volume of testing sample, such as 500 μl testing sample specified in the EN ISO 11348-2007 standard, even less. Hence, the precision instruments used for chemical analysis is capable of preparing the samples for LBTA. Pretreatment methods for traditional LBTA have been developed in recent years.

The method combining the Microtox® test with reverse-phase high performance liquid chromatography (RP-HPLC) fractionation

was successfully applied on the toxicity evaluation (Lei and Aoyama, 2010). The fractions obtained by RP-HPLC were then further analyzed with gas chromatography/mass spectrometry for gaining the possible causes of the toxicity in landfill leachates. Actually, solid phase extraction (SPE) as a pretreatment method of bioassay could also be used to extract target substances and divide the target substances into different characters of fractions (Smital et al., 2011). Contrasting with SPE, passive samplers, including semipermeable membrane devices (SPMDs) and styrenedivinylbenzene-reverse phase sulfonated (SDB-RPS) Empore™ disk, were also proven to be effective in extracting organic substances from the water samples, but it is incapable of obtaining the different characters of fractions (Goodbred et al., 2009; Shaw et al., 2009).

Accumulating inorganic substances from water or solid phase samples for bioassay is much more difficult than organic substances. Diffusive Gradient in Thin-Films (DGTs) composed of a layer of Chelex resin impregnated on hydrogel is suitable to extract metals from the water samples for LBTA (Roig et al., 2011).

Although the ISO 11348 standard for the determination of the inhibitory effect of water samples on the light emission of *A. fischeri* has been widely recognized and applied for a long time, its protocol is hard to prevent the interference caused by turbidity or color of the testing samples. Filtration may be an option for decreasing the turbidity, while the toxicity would be underestimated due to the removal of toxic substances adsorbing on the particles. The new standard ISO 21338:2010 based on the kinetic mode of *A. fischeri* bioassay firstly developed by Lappalainen J. in 1999, was newly issued for evaluating the inhibitory effects of solids and colored samples (ISO, 2010). In this protocol, the light intensity of the control was determined with the peak luminescence measured in the first 30s exposure of the bacteria to the sample for mitigating the drawbacks of the traditional standard protocol (ISO 11348), and hence it was called "Flash" toxicity assay (Kovats et al., 2012a). Comparing with Microtox® system, turbidity and color of the sample will not lead to false toxicity reading in this Flash toxicity system (Kovats et al., 2012b). The Flash assay combining with MTA is also available to obtain a high sample throughput (Kurvet et al., 2011).

A new system named TOXcontrol® Toxicity Monitoring System, was designed and applied for full automatic on-line bioassay of toxic compounds using luminescent bacteria. Comparing with standardized Microtox® assay, it was certified to be accurate and reproducible enough to be used as an on-line automatic warning system (Lopez-Roldan et al., 2012). A flow injection analyzer implementing biosensors was developed for full automatic LBTA of water samples (Komaitis et al., 2010; Pinto et al., 2012). To perform the flow injection analysis of gas toxicity, the luminescent bacteria were trapped on a thin polyion complex membrane, and the testing sample was injected into the test cell to contact with the luminescent bacteria (Komori et al., 2009). These automatic bioassays based on the standard bioluminescence inhibition of *A. fischeri* ensured the precise control of test condition (e.g. exposure time, the volume of testing sample and bacterial suspension), reduced the costs of the assay, and increased the sample throughput. The LBTA is developing toward high automation and reproducibility.

4. Factors affect the performance of LBTA

4.1. Factors affect the toxicity prediction of known chemicals

4.1.1. For single chemicals

Vighi et al. (2009) indicated that the toxicity of narcotics on *A. fischeri* could be accurately predicted with the n-octanol/water partition coefficient (Kow). The model used was simplified as Eq. (3)

$$\log(1/EC_{50}) = a \log Kow + b \quad (3)$$

where a and b are the model parameters. The relationships between the toxicity of narcotics on algae, Daphnia, and fish and Kow also follow this model. Frank et al. (2010) also agreed that Eq. (3) was a good tool for predicting the toxicity of naphthenic acids (NA). Meanwhile, this model could serve to identify the effect of NA structures on the toxicity. Predicting the toxicity of NA with equal molecular weight using this model, NA containing fewer carbon rings could produce an increased toxicity while NA with a linear grouping of carbon rings had a larger toxic effect than NA with the carbon rings in a clustered grouping (Frank et al., 2010). Investigating the toxicity of eight congeneric sets of NA to *A. fischeri*, in different sets, their toxicity related to both the structures and the carbon numbers of NAs, and in the same set, the toxicity increased following the increasing carbon numbers of NAs (Jones et al., 2011). Aruoja et al. (2011) also declared that the toxicity of both anilines and phenols to *A. fischeri* depended on not only the Kow but also the other characteristics of the chemicals.

Recently, the food safety issues regarding the plasticizers have received increasing attention. Researchers resorts to the means of biological response for hazard identification of plasticizers. For the toxicity of dibenzoate plasticizers containing similar structures to *A. fischeri*, a decreasing toxicity of plasticizers was observed following the increasing alkyl chain size (Segura et al., 2012). Therefore, toxicity of chemicals is closely related to the detailed molecular structure, in addition to the chemical parameter Kow.

Ionic liquids (ILs) are a novel class of solvents with the interesting properties, such as very low vapor pressure, robust thermo stability, high ionic conductivity and relatively low viscosity (Yan et al., 2012). In addition, the IL can be easily designed and synthesized in the laboratory to meet special properties by adjusting the types of the anions, the cations and the linked chains of IL (Yue et al., 2011). ILs as alternatives to organic solvents are widely applied in various fields. Many studies were focused on the potential relationships between the toxicity of ILs on luminescent bacteria and the molecular structures of ILs. Ventura et al. (2013) indicated that the ILs could be divided in two groups (the aromatic and the non-aromatic ILs) presenting very different dependencies of the toxicity with the water solubility. A quantitative structure-activity relationships (QSARs) was properly established for predicting the toxicity of 96 ILs to *A. fischeri*, considering the structures of the anion, the cation and the carbon chains linked to the cation (Luis et al., 2010). Moreover, the alkyl chain length had the greatest contribution on the toxicity of ILs (Luis et al., 2010). The toxicity of imidazolium- and phosphonium-based ILs increased with increasing alkyl chain length, whereas the guanidinium-based ILs did not follow this trend (Ventura et al., 2012). The introduction of oxygenated groups on the alkyl chains, such as ether and ester, could lead to a decrease of the toxicity of ILs, normally independent of the cation (Ventura et al., 2012). To investigate the hydrophilic and hydrophobic ILs with low toxicity, such as imidazolium, pyridinium, pyrrolidinium and tropinium, the EC_{50} values had a good liner relationship with the number of aliphatic carbons surrounding pyridinium cations and the logEC_{50} values also had a liner

Table 1
Molecular structures related to the toxicity of chemicals to luminous bacteria presented in articles.

Molecular structures related to toxicity	Chemicals	References
The number of carbon rings and their permutation	NA	(Frank et al., 2010)
Straight-chain, methyl branch, carbon ring, aromatic structure, carbon numbers	NA	(Jones et al., 2011)
The number and position of substituent	Anilines, phenols	(Aruoja et al., 2011)
Alkyl chain size	Plasticizers	(Segura et al., 2012)
Aromatic or non-aromatic structures	ILs	(Ventura et al., 2013)
The anions, the cations and the carbon chains linked to the cation, the alkyl chain length	ILs	(Luis et al., 2010)
Alkyl chain length and the oxygenated groups on the alkyl chains	ILs	(Ventura et al., 2012)
The number of aliphatic carbons surrounding cations and the total number of carbons on cations	ILs	(Viboud et al., 2012)

relationship with the total number of carbons on cations (Viboud et al., 2012). The study on the relationship between toxicity to luminescent bacteria and molecular structures of ILs could not only contribute to predicting the toxicity of untested ILs but also to optimizing the ILs for decreasing the toxicity by adjusting the molecular structures (e.g. the aromatic and aliphatic structure) and components (e.g. the anion, the cation) based on the conditions that meet the application. Table 1 is made to take a rapid glance at that which molecular structures affect the toxicity of chemicals to luminescent bacteria.

However, a parabolic relationship between logKow and $\log(1/EC_{50})$ was observed in a study which investigated both the short-term and long-term toxicity of six herbicides (Zhu et al., 2009). So, the relationship between logKow and $\log(1/EC_{50})$ is not necessarily liner, as is described in Eq. (3). Because the molecular features such as the carbon number of chemicals play more important role than the hydrophobicity. This study also stated that the dynamic analysis of toxicity based time-dependent toxicity assay could better understand the toxicity process of toxicants to organisms.

For the luminescent bacteria assay, many models have tried to fit the relationship between inhibition response of luminescent bacteria and the concentration of chemical (Liu et al., 2013; Teodorovic et al., 2009; Vighi et al., 2009). However, it is a challenge to predict the hormetic effect of chemicals on luminescent bacteria and interpret its mechanism. The most observed hormesis was in the chronic toxicity to luminescent bacteria. Based on the molecular docking and QSARs, Deng et al. (2012) proposed a new piecewise function for fitting the biphasic hormetic concentration-response curve obtaining from chronic toxicity assay with two new parameters and explored the mechanism of the hormesis of sulfonamide antibiotic to *P. phosphoreum*. In the study of the toxicity of ILs to luminescent bacteria, the apparent hormesis of several ILs attracted researchers' attention. It was identified that the hormesis of ILs to Q67 depended on their exposure concentration, exposure time and structures (Wang et al., 2011). With regard to the structure of ILs, side chains had a close relationship with the hormetic effects on Q67 (Zhang et al., 2013).

4.1.2. For chemical mixtures

The contaminants do not exist isolated in the environment. A variety of contaminants have a joint effect on the organisms, and the toxicity of chemicals in a mixture may not coincide with that predicted from the single chemical toxicity. The methods for predicting mixture toxicity are usually based on the two models: concentration addition (CA) and independent action (IA). The mathematical equations for CA and IA are expressed as Eqs. (4) and (5), respectively (Liu et al., 2009).

$$\sum_{i=1}^{n} \frac{c_i}{EC_{xi}} = 1 \tag{4}$$

$$E(c_{mix}) = 1 - \prod_{i}^{n} [(1 - E(c_i)] \tag{5}$$

In Eq. (4), c_i is the concentration of the i-th component in a mixture of n chemicals, and EC_{xi} is the concentration of the i-th component that elicits x% inhibition effect when applied individually. In Eq. (5), $E(c_{mix})$ is the total response of the mixuture and $E(c_i)$ is the response of the i-th component. Logit (Eq. (6)) and Weibull (Eq. (7)) functions usually used to fit the concentration–response data of individual chemicals and mixtures are expressed as follows (Zhang et al., 2012).

$$E = 1/(1 + \exp(-\alpha - \beta \log c)) \tag{6}$$

$$E = 1 - \exp(-\exp(\alpha + \beta \log c)) \tag{7}$$

where, E is the response of the chemicals or mixtures with the concentration of c, and α and β are the model parameters.

Recently, many researchers have focused on predicting the mixture toxicity and identifying the toxicity interaction of herbicides, insecticides and ILs to luminescent bacteria using the CA and IA models. Theoretically, the model of CA is based on the assumption that all mixture components have a similar mechanism of action, while the model of IA is used for predicting the toxicity of mixtures composed of dissimilarly acting chemicals. For the organophosphorus (OP) pesticides, the mixture toxicity of six OP pesticides could be well estimated by the CA model (Zhang et al., 2008). For pesticides, it was concluded that CA was a suitable model for predicting the toxicity of mixtures of chemicals with unspecific mechanisms of action (Zhou et al., 2010). Huang et al. (2011) indicated that both CA and IA models were capable of predicting the mixture toxicity of seven phenolic compounds with similar and dissimilar action mechanisms to Q67. These researchers aimed at founding the predictability of CA and IA models for different chemicals. Actually, Villa et al. (2012) have confirmed the suitability of the two models based on investigating the toxicity of complex mixtures of chemicals with different structures, toxicological modes of action.

Comparing the concentration–response data observed from the experiments with that predicted from the CA and IA models, the toxicity interaction, such as antagonistic, synergistic, CA or IA, could also be identified. This has been applied on analyzing the toxicity interaction of ILs mixtures (Zhang et al., 2011, 2012), the mixtures of ILs and pesticides (Zhang et al., 2009). Tian et al. (2012) demonstrated that the toxicity interactions depended on the number of components, the dominating components and the toxic ratios of individual chemicals.

To predict the toxicity of non-interactive mixture on Q67, a new model that integrated CA with IA was developed and proved to be equivalent or better than the CA and IA models (Qin et al., 2011). Nevertheless, CA and IA models are incapable of predicting the hormesis of mixtures on luminescent bacteria. CA model could not predict the

Table 2
Correlation coefficients of EC_{50} values for luminous bacteria and conventional indexes collected from the published articles.

COD	+0.084 (Bayo et al., 2009); −0.58 (Mendonça et al., 2009); −0.607 (Katsoyiannis and Samara, 2007); − (Ma et al., 2011); 0 (Rodrigues and Umbuzeiro, 2011)
TN	+0.670 (Bayo et al., 2009); + (Ore et al., 2007); −0.642 (sludge) (Roig et al., 2012)
NH_4^+	+0.363 (Bayo et al., 2009); 0 (Ore et al., 2007); + (Wang et al., 2007); +0.72 (sludge) (Ocampo-Duque et al., 2008)
BOD_5	−0.56 (Mendonça et al., 2009); −0.607 (Katsoyiannis and Samara, 2007)
pH	+0.694 (Bayo et al., 2009); 0 (sludge)(Roig et al., 2012)
Chlorine	−0.514 (Bayo et al., 2009); − (Wang et al., 2007)
TOC	−0.163 (Bayo et al., 2009); 0 (Ore et al., 2007)
DOC	− (Macova et al., 2010); − (Wang et al., 2007)
UV_{254}	− (Wang et al., 2007)
TIC	+0.735 (Bayo et al., 2009)
TC	+0.727 (Bayo et al., 2009)
DO	+ (Ma et al., 2012)
SS	+0.592 (Katsoyiannis and Samara, 2007)

+: the EC_{50} values have a significant positive correlation with conventional indexes. −: the EC_{50} values have a significant negative correlation with conventional indexes. 0: the EC_{50} values have no correlation with conventional indexes. DOC: dissolved organic carbon. UV_{254}: the absorbance of ultraviolet at 254 nm.

stimulatory section below the lowest stimulatory effect of individual chemicals (Ge et al., 2011). Theoretically, IA model will lose its probabilistic meaning in stimulatory section. Thus, Zou et al. (2013) proposed a new efficient model named as "six-point" to predict the hormesis of mixtures whether it is interactive or non-interactive.

4.2. The correlation of conventional indexes with toxicity

Bayo et al. (2009) determined the acute toxicity of secondary effluent from wastewater treatment plants (WWTPs) after a chlorination–dechlorination process. It was illustrated in this study that the toxicity of wastewaters to *A. fischeri* had: (i) a strong positive correlation with chlorine levels, (ii) a strong negative correlation with TN, total inorganic carbon (TIC), TC, and pH, and (iii) an insignificant correlation with COD and TOC. An empirical model was then properly developed for predicting the toxicity of actual wastewaters to *A. fischeri* based on binary quadratic polynomial of TN and TC. Therefore, the toxicity of actual samples to luminescent bacteria may have a potential correlation with the conventional indexes.

Taking COD into account, Rodrigues and Umbuzeiro (2011) also showed that the toxicity of treated effluent to *A. fischeri* was not relevant to its COD. However, significant negative correlations were found between the EC_{50} values of wastewaters from sewer network systems or WWTPs and the parameters of COD (Katsoyiannis and Samara, 2007; Mendonça et al., 2009). As such, it can be concluded that the toxicity of wastewater in a high level, such as untreated wastewater, may show an apparent correlation with its COD, while the toxicity of wastewater in a low lever, such as treated effluent, may show an inconspicuous correlation with its COD. In contrast with COD, TOC as another parameter of organic load may have insignificant correlation with the parameter of toxicity (Ore et al., 2007).

For the nitrogen content, there was already compelling evidence that the toxicity of wastewater to luminescent bacteria is associated with TN and NH_4^+ (Bayo et al., 2009). Most of studied showed that the toxicity decreased with the increase in TN or NH_4^+ (Ore et al., 2007; Wang et al., 2007). On the contrary, Roig et al. (2012) revealed that the total nitrogen of sewage sludge from WWTPs had a significant negative correlation with its EC_{50} value obtained from Microtox® test of aqueous extract. This indicated that the toxic effect of nitrogen-containing compounds in wastewater was different from that in sludge.

In the WWTPs, disinfection is usually as the final treatment for the inactivation of harmful pathogens and microorganisms. It has been known that most of disinfection by-products (DBPs) produced in disinfection process had adverse effect on animals and humans (Hebert et al., 2010; Shen et al., 2010). The toxicity of secondary effluent increased after chlorine disinfection (Pignata et al., 2012). Actually, the toxicity of disinfected wastewater to luminescent bacteria had a strong positive correlation with the concentration of chlorine (Wang et al., 2007; Bayo et al., 2009).

Based on the QSARs of chemicals, it could be obtained that the toxicity to luminescent bacteria is related to the chemical structures, such as the aromatic or aliphatic structures. For actual wastewater, the toxicity to *P. phosphoreum* was also proved to have a positive correlation with the parameter of UV_{254} expressed the degree of aromaticity of organic substances in water (Wang et al., 2007).

Concerning surface waters, the concentration of dissolved oxygen (DO) is an important parameter for indicating the healthy state of water body. Under anaerobic condition, the organic substances could produce high toxic intermediate products. Thus, it is reasonable that the toxicity has a negative correlation with the concentration of DO (Ma et al., 2012).

Toxicity of actual wastewater or sludge to luminescent bacteria is affected by many factors. The correlations between toxicity and the physicochemical parameters are determined by the characteristics of wastewater or sludge. Those above reported and more information, concerning the correlations between EC_{50} value in acute toxicity assay using luminescent bacteria and conventional indexes, are summarized in Table 2 for showing the overall of their correlations.

5. Comparing the sensitivity of LBTA with other bioassays

To correctly analyze the results of LBTA, the sensitivity of luminescent bacteria needs to be discussed for different chemicals or samples. Table 3 presented the sensitivity of acute toxicity assay using

Table 3
The comparison of the sensitivity of acute *A. fischeri* assay with other bioassays for the different testing samples.

Sample description	Organisms	Sensitivity of acute *A. fischeri* assay	Reference
Cadmium; zinc; manganese; lead.	*A. fischeri*; *Pseudomonas putida*; *D. magna.*	Not the most sensitive to these four heavy metals	(Teodorovic et al., 2009)
Fosthiazate; metalaxyl-M; imidacloprid; Cu.	*A. fischeri*; *Pseudokirchneriella subcapitata*; *D. magna.*	The most sensitive to fosthiazate and metalaxyl-M.	(Kungolos et al., 2009)
PAMAM Dendrimer G-4, G-5, and G-6.	*A. fischeri*; *D. magna*; *Thamnocephalus platyurus*; *PLHC-1* and *RTG-2* fish cell lines.	Less sensitive than *D. magna* test.	(Naha et al., 2009)
The new natural formicide Macex®	*A. fischeri*; *D. magna*; *P. subcapitata*; *T. platyurus*; *Heterocypris incongruens*; *LuminoTox* (photosynthetic enzyme complexes).	*P. subcapitata* ≈ *D. magna* ≈ *A. fischeri* > *T. platyurus* > *LuminoTox* > *Heterocypris incongruens*	(Burga-Perez et al., 2013)
Azo dyes	*Comet assay*; *Salmonella; cell viability*; *Daphnia similis*; *A. fischeri.*	No toxic effects were observed for *A. fischeri.*	(Ferraz et al., 2011)
Antibiotics	*A. fischeri*; *P. subcapitata*; *Microcystis aeruginosa*; *Nouws* antibiotic test.	Antibiotics have no effect on *A. fischeri.*	(van der Grinten et al., 2010)
The wastewater containing amoxycillin after anaerobic treatment	*A. fischeri*; *D. magna*; *Lepistes sp.*	*A. fischeri* is the most sensitive organisms.	(Celebi and Sponza, 2012)
The wastewater produced in the photocatalytic degradation process of wastewater contaminated with cyanide and phenol	*A. fischeri*; *Polystichum setiferum spore.*	More sensitive than fern spore.	(Marugan et al., 2012)
Wastewaters from municipal sewer networks and respective WWTPs.	*A. fischeri*; *P. subcapitata*; *T. platyurus*; *D. magna*; *Lemna minor.*	*A. fischeri* > *T. platyurus* > *D. magna* = *P. subcapitata* > *Lemna minor*	(Mendonça et al., 2009)
Pharmaceutical wastewater containing kemicetine	*P. phospherium; D. magna; Chlorella.*	*P. phosphoreum* and *D. magna* have the similar high sensitivity to the pharmaceutical wastewater.	(Sponza and Demirden, 2010)
Industrial and municipal wastewaters	*A. fischeri*; *D. magna*; *Tetrahymena thermophilla.*	Stimulating effect to *A. fischeri* was detected in the measurement of wastewater from food industries.	(Soupilas et al., 2008)
Soil aged for 69 days and the soil spiked with pyrene	*Brassica rapa*; *Eisenia fetida*; *A. fischeri.*	The most sensitive indicator of pyrene	(Khan et al., 2012)

Table 4
Application of LBTA on environmental samples.

	References
Water	
Biological wastewater treatment process	(Celebi and Sponza, 2012; Huang et al., 2010; Katsoyiannis and Samara, 2007; Reginatto et al., 2009; Saddoud et al., 2009; Sponza and Demirden, 2010)
Advanced oxidation wastewater treatment process	(Daghrir et al., 2013; Haidar et al., 2013; Hsu et al., 2013; Kawabata et al., 2012; Marugan et al., 2012; Rosal et al., 2009; Shemer and Linden, 2007)
Advanced oxidation wastewater treatment coupled with other treatment process	(Amat et al., 2009; Cotman and Gotvajn, 2010; De Schepper et al., 2012; Macova et al., 2010; Perdigón-Melón et al., 2010; Zapata et al., 2009; Zhao et al., 2010)
Others	(Bayo et al., 2009; Kovats et al., 2012a; Lei and Aoyama, 2010; Macken et al., 2008; Pignata et al., 2012; Rodrigues and Umbuzeiro, 2011; Roig et al., 2011; Shaw et al., 2009; Shen et al., 2010; Soupilas et al., 2008; Wang et al., 2007; Ye et al., 2011)
Solid	
Sediments	(Burga Pérez et al., 2012; Guerra et al., 2007; Karlsson et al., 2010; Linsak et al., 2012; Mamindy-Pajany et al., 2012; Mamindy-Pajany et al., 2013; OcampoDuque et al., 2008; Piou et al., 2009)
Wastes	(Deprez et al., 2012; Ore et al., 2007; Chou et al., 2009)
Sludge	(Batziaka et al., 2008; Katsoyiannis and Samara, 2007; Roig et al., 2012; Samaras et al., 2008; Silva et al., 2011)
Soil	(An et al., 2012; Hirmann et al., 2007; Khan et al., 2012; Maisto et al., 2011; Mekki et al., 2008)
Air pollutants	(Cukurluoglu and Muezzinoglu, 2013; Kovats et al., 2012b; Roig et al., 2013; Turoczi et al., 2012; Vouitsis et al., 2009)

luminescent bacteria for the different test samples, comparing with other bioassays.

A. fischeri was not the most sensitive species to heavy metals (Cd, Zn, Mn, Pb) in comparison with *Pseudomonas putida* and *Daphnia magna* (Teodorovic et al., 2009). Determining the toxicity of fosthiazate, metalaxyl-M, imidacloprid and Cu using *A. fischeri*, *Pseudokirchneriella subcapitata* and *D. magna*, showed that *A. fischeri* was the most sensitive to fosthiazate and metalaxyl-M (Kungolos et al., 2009). Comparing with *D. magna*, *Thamnocephalus platyurus*, *PLHC-1* and *RTG-2* fish cell lines test, *A. fischeri* is only less sensitive than *D. magna* when using to measure PAMAM Dendrimer G-4, G-5, and G-6 (Naha et al., 2009). Concerning the new natural formicide Macex®, the sensitivity of *A. fischeri* was similar with *D. magna* and *P. subcapitata* and better than *T. platyurus*, *Heterocypris incongruens*, *LuminoTox* (photosynthetic enzyme complexes) (Burga-Perez et al., 2013). Many studies have indicated that azo dyes widely used in textile dyeing are often associated with carcinogenicity and mutagenicity (Caritá and Marin-Morales, 2008; Chequer et al., 2009), while there were no indication of toxicity of azo dye Disperse Orange 1 to *A. fischeri* (Ferraz et al., 2011). Antibiotics can be targeted to inhibit or kill pathogenic microorganisms (Mojica and Aga, 2011; Dong et al., 2012). Celebi and Sponza (2012) observed that among three trophic organisms, *A. fischeri* was the most sensitive one to the wastewater containing amoxycillin after anaerobic treatment. However, antibiotics, including sulphamethoxazole, trimethoprim, flumequine, tylosin tartate and streptomycin sulfate and oxytetracycline dehydrate, had no toxic effect on *A. fischeri* (van der Grinten et al., 2010). Hence, *A. fischeri* was selective to respond to the antibiotics.

Based on the study of Marugan et al. (2012) on the variation of toxicity of wastewater containing cyanide and phenol in the photocatalytic degradation process, *A. fischeri* was much more sensitive than fern spore. From measuring the toxicity of wastewaters from municipal sewer networks and WWTPs with different trophic organisms, it was found that *A. fischeri* was the most sensitive organisms among *P. subcapitata*, *T. platyurus*, *D. magna* and *Lemna minor* (Mendonça et al., 2009). Comparing *P. phospherium*, *D. magna* and *Chlorella*, *P. phosphoreum* and *D. magna* had the similar high sensitivity to the pharmaceutical wastewater (Sponza and Demirden, 2010). *A. fischeri* was not suitable to evaluate the toxicity of wastewaters from food industries due to the stimulating effect (Soupilas et al., 2008). Khan et al. (2012) detected the toxicity of soil contaminanted by pyrene using a series of bioassay, suggesting that *A. fischeri* was the most sensitive bio-indicator of pyrene.

From the above discussion, it can be seen that for most samples, *A. fischeri* was sensitive enough to be employed in toxicity assay. However, some contaminants have a selective detrimental effect on organisms. Due to the limitation of trophic level, some contaminants could not show any toxic effect on luminescent bacteria, such as Disperse Orange 1 and some antibiotics. A series of bioassays using organisms from different trophic levels is usually recommended for evaluating the toxicity of samples. These previous studies could provide a guidance to choose the suitable organisms for toxicity assessment of chemicals and to properly analyze the results of toxicity assay.

6. The application of luminescent bacteria on environmental monitoring

6.1. For water samples

The toxicity of water samples is easier to be tested by LBTA than that of solid and gas phase samples. The bioassay based on luminescent bacteria can be useful employed in monitoring the treated effluent and optimizing the operational parameters of wastewater treatment processes.

An anaerobic-anoxic-oxic (A2/O) processes was optimized for reducing the toxicity of municipal wastewater to *P. phosphoreum*, which indicated that the toxicity could reduce by 82.2% under the optimal condition (Huang et al., 2010).

The Microtox® test was conducted to determine the variation of toxicity of urban wastewater during a day (Saddoud et al., 2009). The results showed that the toxicity tended to increase during a day, which was associated with the intensity of industrial activity in a day.

A test battery, with the aim to represent the baseline toxicity using *A. fischeri* toxicity assay and the specific toxicological modes of action with five additional bioassays, was applied to evaluate the removal efficiency of organic micropollutants in the advanced wastewater treatment processes, suggesting that the coagulation/flocculation/dissolved air flotation/sand filtration and the ozonation/activated carbon filtration processes were the most effective to removal the toxicity of organic micropollutants (Macova et al., 2010).

A. fischeri toxicity assay in combination with other bioassays and COD parameter was applied to evaluate and optimize the sequential ozonation-biological treatment process (O_3-Biodegradation-O_3-Biodegradation) and the conventional partial ozonation process (O_3-Biodegradation), demonstrating the superiority of sequential ozonation-biological treatment process (De Schepper et al., 2012).

The toxicity of pectin production wastewater was investigated by means of *A. fischeri* and *Scenedesmus subspicatus* bioassays, and a series of chemical analysis were then performed to determine the chemical characteristics of the samples (Reginatto et al., 2009). The results showed that the toxicity of pectin production wastewater was related to the polyaromatic organic matter in wastewater.

The methods, including air stripping, adsorption on activated carbon and zeolite and Fenton oxidation, were conducted for evaluating the efficiency of toxicity removal of landfill leachate using activated sludge

and *A. fischeri* (Cotman and Gotvajn, 2010). Adsorption to powdered activated carbon and zeolite could effectively reduce the toxicity of landfill leachate.

Three marine species: *A. fischeri*, *Tetraselmis suecica*, and *Tisbe battagliai*, were employed to assess the acute toxicity of five organic contaminants frequently detected in marine sediments and in Toxicity Identification Evaluation (TIE) (Macken et al., 2008). The marine TIE manipulations, including addition of ethylene diamine tetraacetic acid, addition of sodium thiosulphate, and sample passed through C_{18} column, were performed on spiked tributyltin chloride (TBT-Cl) samples. The results revealed that C_{18} resin was the most effective manipulation for removing the toxicity of samples contaminated by TBT-Cl.

On the other hand, luminescent bacteria acute toxicity assay are usually conducted to evaluate the special treatment processes applied on refractory contaminants or wastewaters and the toxicity of their intermediate products. The application of a treatment process could not only be evaluated by the efficiency of contaminants removal but also by the toxicity of treated chemicals or wastewaters to organisms. The special treatment processes collected from published articles include Fenton, ozonation, UV and electrochemical degradation.

A combined solar photo-Fenton with biological treatment was applied on the degradation of a mixture of five commercial pesticides (Zapata et al., 2009). The parameters of active ingredients, DOC, COD, and *A. fischeri* acute toxicity were used to evaluate the performance of this process. The results of *A. fischeri* assay indicated that the toxicity of pesticides was decreased by the solar photo-Fenton treatment following the decrease of DOC (Zapata et al., 2009). Amat et al. (2009) stated that although the toxicity based *A. fischeri* decreased along the treatment, the remaining toxicity was still significant due to the higher sensitivity and specific nature of *A. fischeri*. The coupled coagulation-Fenton process was also effective to treat cosmetic industry wastewater and to decrease its acute toxicity to *A. fischeri* (Perdigón-Melón et al., 2010).

A. fischeri and *D. magna* were employed to evaluate the variation of toxicity during the degradation of clofibric acid by catalytic ozonation (TiO_2-O_3). With the identification of oxidation by-products and the structure assignment, the increased toxicity in the initial stage of ozonation was attributed to the formation of ring-opened structure (Rosal et al., 2009). The similar change of toxicity to *A. fischeri* in the initial stage of ozonation was also found during the ozonation of microcystins process without or with a lower concentration of TiO_2 (Hsu et al., 2013).

The method combing *A. fischeri* toxicity assay with chemical analysis was employed to study the structure and toxicity of ultraviolet-photoproduct of acetaminophen (AA) (Kawabata et al., 2012). The toxicity of photo-exposed AA solution increased due to the formation of by-product (Kawabata et al., 2012), which coincided with the photodegradation of polycyclic aromatic hydrocarbons (Shemer and Linden, 2007). In case of oxytetracycline, the toxicity of oxytetracycline solution to *A. fischer* increased after UV photodegradation, while being decreased in the presence of TiO_2/5A zeolite and TiO_2/13X zeolite due to the adsorption of zeolite (Zhao et al., 2010).

Microtox® test was applied on the toxicity assessment of the electro-oxidation of sulfachloropyridazine (SCP) solutions, showing that the toxicity of SCP solutions increased because of the formation of more toxic compunds (Haidar et al., 2013).

Hence, in most cases the advanced oxidation treatment, such as O_3 or UV oxidation, usually lead to the toxicity increasing due to the formation of more toxic intermediate products or by-products in contrast with the parent compounds. However, the advanced oxidation process combing with other treatment, such as the biological treatment and adsorption of zeolite, could obtain a desired result of toxicity assay.

6.2. For solid samples

At the beginning, the bioassay based on luminescent bacteria was initiated to assess the acute toxicity of water samples. In spite of the widely and efficient use of LBTA in water samples, its direct application on solid samples is defective and restricted, which has been discussed in Section 3.1. The application of new technology mentioned in this paper on solid samples is less proven.

The operation for investigating the contamination of solid environmental samples is more complicated than that of water samples. Whether for chemical analysis or for biological analysis of solid samples, the manipulation of extraction is commonly required to transfer the contaminations from solid phase to liquid phase. Use of different solvents in extraction process could obtain the extracts containing different target chemicals. For instance, an aqueous extract would contain heavy metals and hydrophilic organic substances. Hence, it provides a convenience to analyze the organic or inorganic substances separately.

The Microtox® test was applied to assess the toxicity of aqueous and organic extracts of river sediments. As expected, the toxicity of aqueous extracts had a good correlation with the concentration of heavy metals in sediments. The toxicity of organic extracts was attributed to the presence of persistent organic pollutants and the heavy metals bounded onto the sediments (Ocampo-Duque et al., 2008). Moreover, Batziaka et al. (2008) stated that the toxicity of aqueous extract was significantly associated with the pH in the leaching test.

For risk analysis, both chemical analysis and bioassays are conducted for comprehensively estimating the influence of contaminants on the environment. A new framework was developed for the classification of hazardous wastes using a battery of chemical and biological analysis (Deprez et al., 2012). In this framework, Microtox® test as a general toxicity screening after chemical analysis and before a series of mechanistic toxicity assays was proven to be the most sensitive method to evaluate the acute toxicity of the organic fraction of wastes (Deprez et al., 2012). In addition, the contaminants having the acute toxic effect on luminescent bacteria mostly showed a chronic toxicity in mechanistic toxicity assays (Deprez et al., 2012). While, to classify the industrial sludge as hazardous or non-hazardous, the results obtained from the chemically classification did not conform to that obtained from the ecotoxicity assays (Silva et al., 2011). Linsak et al. (2012) who evaluated the quality of marine sediments using chemical analysis and Microtox® test also suggested that the concentration of contaminants could not stand for the potential toxicity to organisms. The study, with the aim to discuss the correspondence between the concentration of heavy metals in soil and their toxicity by chemical analysis and a battery of bioassays, indicated that the toxicity of soil, with high concentration of heavy metals, to *A. fischeri*, was not only related to the high total concentration of heavy metals in soil but also to the high availability of heavy metals (Maisto et al., 2011). Therefore, it is biased to evaluate the quality of a sample only using chemical analysis or biological analysis.

Chemical sequential extraction and bioassay including the Microtox® solid phase test and embryo-toxicity test on *oyster larvae* were conducted for investigating the arsenic mobility, bioavailability and toxicity in marine port sediments. The results showed that the Microtox® solid phase test was suitable to evaluate the toxicity of As-contaminated sediments, and the toxicity of As(V) to *A. fischeri* and *oyster larvae* was higher than that of As(III) (Mamindy-Pajany et al., 2013).

In these years, the atmospheric particulate matter with aerodynamic diameter less than 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$) attract researchers' attention due to their hazardous to human health. In cities, transportation is a major contributor to particulate matter (PM). Vouitsis et al. (2009) investigated the characters of PM emitted from different light-duty vehicles using chemical analysis and Microtox® tests. The results showed that on a per mass basis of all tested samples, the gasoline vehicle PM showed higher toxicity than that of conventional diesel vehicle and the diesel vehicle equipped with a catalyzed diesel particle filter (Vouitsis et al., 2009). Microtox® test in combination with a series of *in vitro* test were used to assess the toxicity of PM_{10} obtained with air filter, indicating that Microtox® test was a robust method to assess the air pollution (Roig et al., 2013). Kovats et al. (2012b) developed a direct contact test to estimate the toxicity of

aerosol based on "Flash" toxicity assay and showed its advantages on assessing aerosol toxicity. Appling this method on urban particulate matter, Turoczi et al. (2012) suggested that the toxicity of PM to *A. fischer* was greatly associated with its sources and seasonality. *A. fischer* toxicity assay was employed to evaluate the toxicity of dry and wet atmospheric deposition samples and six heavy metal solutions (Cukurluoglu and Muezzinoglu, 2013). The results showed that the average toxicity value of the wet deposition samples collected during rainfall was 15% higher than that of dry deposition samples collected without rainfall, and the heavy metals showed an antagonistic effect in depositions no matter dry or wet (Cukurluoglu and Muezzinoglu, 2013). Hence, the influence of the state (dry or wet) of atmospheric deposition on its toxicity was not pronounced. The application of LBTA on atmospheric deposition is a new research direction.

All the papers reported in the above Sections 6.1 and 6.2 and more papers concerning the application of LBTA on environmental samples are listed in Table 4.

7. Future perspectives and research needs of LBTA

The characters of luminescent bacteria that are short reproduction cycle, easy to be cultivated and to be observed, drive the luminescent bacteria inhibition assay to become the first choice of the biological toxicity assay. The LBTA is rapid, efficient and cost effective, which has been emphasized by many studies before.

The traditional LBTA reflects the general acute toxicity due to its low selectivity. Now the protocol of this assay is going to be miniaturization for reducing the costs and increasing the throughput. The traditional LBTA combing with the special pretreatment conducted with precise instrument can improve the selectivity of the toxicity detection. The bioassay based luminescent bacteria is moving toward high automation and reproducibility because of the application of flow injection technology and biosensor. Nevertheless, the full automatic LBTA needs to be commercialized for proving its good performance and expanding its application. Furthermore, the dynamic analysis of chronic toxicity assay using luminescent bacteria could gain the impact of contaminants on bacteria over the whole bacterial cycle life. The time of the chronic toxicity assay using luminescent bacteria is much shorter than that using other organisms.

It can be seen from above discussion that a large part of interferences are from the complex matrices of testing samples. By means of choosing an appropriate pretreatment methods and improving the detection system, the interferences could be decreased and the results of LBTA could be more authentic and reliable. Based on a vast experimental data, establishing the QSARs of chemicals and analyzing the joint effects of mixtures can help to describe the mechanisms of chemicals to luminescent bacteria, to obtain the toxicity results of untested samples and to determine the toxicity interaction of mixtures. Although the hormesis has been observed long before, to date, the studies focused on predicting the hormesis of chemicals and its impact factors emerged in recent years is still not satisfactory. The toxicity of chemicals to luminescent bacteria is closely related to the structures and physicochemical characters of chemicals. To develop a model for predicting the toxicity of chemicals is a profitable direction of future research on LBTA. Owing to the complexity of actual samples, the correlation between the toxicity based luminescent bacteria and conventional indexes is indeterminate, which needs further research. The application range of LBTA is now being expanded, such as the toxicity evaluation of air pollutants using luminescent bacteria.

8. Conclusions

Due to its various advantages, the traditional LBTA has been standardized and proven to be a robust approach for monitoring the environmental pollution. The continuous development of LBTA improves the traditional method, and expands the application range. The toxicity to luminescent bacteria depends on the structure, components and physicochemical characters of chemicals and the characteristics of actual samples. In spite of high sensitivity of luminescent bacteria, the bioassay based on the luminescent bacteria cannot be sensitive to any samples. A battery of bioassays based on the different trophic levels is usually required for assessing the ecotoxicity of actual samples. The bioassays in combination with chemical analysis are recommended for comprehensive evaluation, as the toxicity results do not coincide with the results of chemical analysis in most cases.

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