Journal of Environmental Monitoring

Cite this: *J. Environ. Monit.*, 2011, **13**, 2914

www.rsc.org/jem

PAPER

Whole-cell bacterial biosensors for rapid and effective monitoring of heavy metals and inorganic pollutants in wastewater

Ademola O. Olaniran,* Lettisha Hiralal and Balakrishna Pillay

Received 13th January 2011, Accepted 2nd August 2011
DOI: 10.1039/c1em10032g

The increasing number of potentially harmful pollutants in the wastewater effluent discharge necessitates the need for the development of fast and cost effective analytical techniques for extensive monitoring programmes to assess the effectiveness of the treatment process. This study compared the use of bacterial biosensors to the conventional *Daphnia magna* assay, Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) tests as well as chemical analysis, for monitoring the toxicity of wastewater. The bacterial biosensors constructed in this study, using *S. sonnei* and *E. coli*, were found to be sensitive to the toxicity of the wastewater effluents. A linear increase in bioluminescence with increasing concentration of heavy metals and inorganic pollutants in water was observed, with a correlation coefficient (r^2) as high as 0.995 and 0.997, respectively. No notable correlation between biosensor toxicity and BOD and COD test results was observed. These bacterial biosensors could provide appropriate alternatives for a rapid, sensitive and cost effective detection of wastewater quality. However, the differences in sensitivity obtained for the different systems suggest that the use of a battery of toxicity assays may be required to provide a real ecotoxicological assessment of wastewater samples.

Introduction

Wastewater treatment plants (WWTPs) are an important component of the wastewater infrastructure and receive wastewaters from homes, commercial buildings, hospitals, *etc.*, for treatment. In addition to domestic sewage, industrial effluents are sometimes also discharged into WWTPs for further treatment before being released into the environment; although some industrial facilities are equipped with their own wastewater treatment processes.[1] The activated sludge process is considered the most efficient method for the biological treatment of wastewaters. The process is based on the development of a heterogeneous microbial community in the aeration basin, which allows the system to be flexible, with regard to considerable fluctuation in the influent wastewater composition.[2] Thus, microbial activity and population are crucial for the effective operation of the system.

Toxic influent is one of the major causes of the failure of biological treatment plants as the presence of a high concentration of certain toxic substances in the influent may result in the reduction of microbial activity, subsequently resulting in non-compliance with discharge permit limits.[2,3] Similarly, gross discharges of untreated or insufficiently treated wastewater effluent into receiving waters are generally considered to be one of the most significant causes of water pollution.[4] Such process upsets may be avoided if the influent and treated wastewater effluents are monitored for toxicity and appropriate protective actions taken when toxicity is detected.[1]

Discipline of Microbiology, School of Biochemistry, Genetics and Microbiology, Faculty of Science and Agriculture, University of KwaZulu-Natal (Westville Campus), Private Bag X54001, Durban, 4000, Republic of South Africa. E-mail: olanirana@ukzn.ac.za; Fax: +27 31 260 7809; Tel: +27 31 260 7400/7401

Environmental impact

Detection of pollutants in wastewater effluents continues to pose a serious environmental challenge, worldwide, owing to the increasing amount of potentially harmful pollutants found in these effluents. This study evaluated the effectiveness of two bacterial biosensor constructs for detecting heavy metals and inorganic pollutants in wastewater. Compared to the conventional tests, bacterial biosensors developed in this study were found to be sensitive to the toxicity of the wastewater effluents. The sensitivity level of these bacterial biosensors to the toxicants tested is promising for a simple, rapid, and cost effective detection and estimation of the concentrations of these pollutants in contaminated water. This could complement other toxicity assays to provide a real assessment of the environmental risk of wastewater effluents.

Traditionally, the quality of treated wastewater is defined by the measurement of parameters such as Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), which reflects the level of organic pollutants in water. However, the conventional analysis based methods alone have been found to be inadequate for the evaluation of the toxicity properties of wastewaters and for ensuring that the influents will not have significant effects on the receiving sewage works.[5] In addition, the standard BOD test involves a complicated and a time-consuming procedure, including a 5-day incubation, and also requires considerable experience and skill to get reproducible results,[6] while the COD test is limited because of its inability to differentiate between biodegradable and biologically inert organic matter.[4] In the past few years, the water flea *Daphnia magna* has become a suitable test organism to continuously monitor water quality by observing differences in the swimming activity of *Daphnia*. Although, the sensitivity of this method is high, the test system sometimes encounters problems in recording the swimming behaviour and requires a longer reproductive cycle and sensing time.[7,8] To overcome these problems, a method that allows for a rapid, cost effective, sensitive and on line monitoring needs to be developed.[6] Although heavy metals are naturally present both in the environment and in water, the anthropogenic activity increases the concentration of metals found in the natural environments.[9] Industrial wastes are therefore mainly responsible for the current pollution of rivers by heavy metals that are normally collected and treated by WWTPs. In addition, heavy metals are easily absorbed by the living organisms because of their great solubility in water, and are found accumulated in humans at the end of the food chain.[10] Thus, there is a need to constantly monitor the level of heavy metal pollution in both the influent and the effluent wastewater to determine the level of treatment required and assess the risk of possible water pollution.

The advent of recombinant DNA techniques for engineering microbial cells has led to interesting possibilities to generate specific microbial sensors.[11] Thus, the use of microbial biosensors for wastewater quality monitoring is rapidly becoming popular mainly due to their sensitivity and ability to provide a real time measurement of the bioavailable fraction of substances and chemicals as opposed to the total concentration.[11] It is also suitable for field work as it does not require expensive and fragile equipment for storage, daily cultivation, handling or specialized training that most analytical methods require.[12] Bacterial luminescence is directly related to cell respiration and is therefore a convenient measure of cellular activity. However, sensitivity of each test species depends on the specific chemical compounds in the sample, therefore, the selection of appropriate indicator organism is critical.[5]

It is always difficult to perform a complete ecotoxicological evaluation of the wastewater effluents because they contain a very complex and variable mixture of compounds. The composition and bioavailability of chemicals within effluents can be highly variable due to degradation and/or chemical interactions with the effluent composition or manufacturing processes.[4] Several bioassays utilizing microorganisms have been described, such as bioassays based on microbial transformations,[13] on the determination of the activity of microbial enzymes,[14] or on the measurement of oxygen consumption using a respirometer.[15] Many of these constructs require a promoter on the plasmid to obtain high expression[8] and could only detect the toxicity of either the wastewater influent or the treated effluent. The need to have an indicator system which can rapidly assess the toxicity of effluents and respond appropriately to all toxicants in wastewater has led to further research into toxicity tests which are rapid, easy and inexpensive. This study therefore aims at developing a bacterial biosensor without a promoter that is both rapid and sensitive for testing the toxicity of both the influent wastewater and the treated effluent for potential use in monitoring the toxicity throughout the treatment facility. South Africa has numerous wastewater treatment plants and the amount of toxicants present in the influent and the effluent is a cause for concern. This study therefore investigated the efficiency and sensitivity of whole-cell bacterial biosensors constructed using *E. coli* and *S. sonnei* for wastewater quality monitoring, based on their luminescence response, compared to the conventional BOD, COD and *Daphnia* tests. This could provide relevant information on the efficiency of the wastewater treatment process as well as serve as an early warning signal on the toxicity level of wastewater effluent samples before being released into the environment.

Experimental

Sample collection and storage

Influent and effluent wastewater samples were collected from two wastewater treatment plants designated as WWTP1 and WWTP2 monthly on three separate occasions. Samples were collected in sterile 5 L plastic bottles, closed tightly and stored at 4 °C prior to use.

Bacterial strains and culture conditions

The bacterial strains used for the construction of the biosensor systems used in this study, *Escherichia coli* and *Shigella sonnei*, were obtained from the culture collection of the Department of Microbiology, University of KwaZulu-Natal (Westville Campus), Durban, South Africa. The isolates were grown and maintained on LB agar slants as described elsewhere.[16]

Construction of bacterial biosensors

Electrocompetent cells of both *S. sonnei* and *E. coli* were successfully prepared using an electroporation method, following standard procedures.[17] The competent cells were transformed with the *pLUX* plasmid, harbouring the *Lux*-CDABE operon using the Gene Pulser apparatus (BioRad) set at 25 μF and 2.5 kV for 0.2 cm cuvettes (BioRad) and the Pulse controller (BioRad) set at 200 Ω as previously described.[11] Recombinant cultures of both organisms were obtained by plating out transformants on LB agar supplemented with 100 μg ml^{-1} of ampicillin.

Toxicity monitoring with bacterial biosensors

Bacterial biosensor cells were grown in a 3 ml LB/amp broth overnight at 28 °C. Thereafter, 500 μl of the pre-culture was added to a 20 ml LB/amp broth and grown to reach an overload of 1×10^6 Relative Light Units (RLUs) by monitoring bioluminescence using a Bio-Orbit 1254 luminometer. Cells were then centrifuged

at 13 000 rpm for 2 minutes and the pellet re-suspended in 3 ml of 0.1 M KCl. Two hundred and seventy microlitres of the appropriate dilutions of the toxicants (effluent samples, heavy metals and inorganic compounds) were used to inoculate the wells of white round-bottomed, 96-well microtitre plate (Nunc, Thermo-Labsystems), followed by the addition of 30 μL of the biosensor cells. Bioluminescence was measured periodically as RLUs using a temperature-controlled Fluoroscan Ascent FL (Thermo-Labsystems) with 15 minute integration time for 1 hour. The toxicity effect of each toxicant was determined by calculating the percentage inhibition (I) of bioluminescence using the following equation: $I = [(A - B)/A] \times 100$ where A = bioluminescence response in the absence of the toxicant (control) and B = bioluminescence response in the presence of the toxicant.

BOD and COD measurements

The biochemical oxygen demand (BOD_5) of the samples was determined using the OxiDirect BOD system (Hach) over a 5 day period. Wastewater samples were pre-treated (*i.e.* adjusting to a neutral pH and filtering) before conducting the analysis, using appropriate volume for the selected BOD_5 range. The chemical oxygen demand (COD) of each sample was determined photometrically using the SpectroQuant Nova 60 COD cell test (Merck) after digestion in a thermoreactor (Hach) at 148 °C for 2 h, following the manufacturer's instructions.

Heavy metal and inorganic compounds concentration

Concentrations of four heavy metals, cadmium, lead, arsenic and mercury, were measured in each sample using an inductively coupled plasma-optical emission spectrometer (ICP-OES) 5300 DV and 2100 DV (Perkin-Elmer). Appropriate heavy metal standard solutions were prepared from stock solutions of each metal in a final volume of 25 ml. Each standard solution (multi-element) was prepared by adding the appropriate amount of each heavy metal from their stock solutions (Merck CertiPUR) to sterile double distilled water in the volumetric flask to achieve the desired concentration followed by the addition of a few drops of nitric acid (HNO_3) for metal preservation. A 20 ml water sample was filtered through 0.45 μm pore size membrane filters to remove any fibers that may cause a blockage in the autosampler of the ICP-OES. The prepared heavy metal standard solutions were used to generate calibration curves from which the concentrations of each metal in the water samples were determined. Prior to analysis, the instrumental parameters (nebulizer argon flow rate and incident power) were optimized followed by the selection of analytical lines free of spectral interferences. The method was validated using biological certified reference materials. Inorganic compound (ammonia and phosphate) concentrations were analyzed by the standard photometric method[18] using the Spectroquant Nova 60 (Merck Pty Ltd) according to the manufacturer's instructions, while the pH was measured by pH meters (Hanna).

Daphnia test

The *Daphnia magna* bioassay was carried out using dormant eggs and salts for preparation of standard freshwater (ISO formula) contained in the "Daphtoxkit F™" (Microtests Inc., Belgium). Hatching of ephippia and preparation of standard freshwater were performed according to the manufacturer's instructions. The ephippia were transferred to hatching Petri dishes with 50 ml pre-aerated standard freshwater, thereafter covered and incubated for 72 h, at 20–22 °C under continuous illumination. Subsequently, the neonates were fed and transferred to standard freshwater with a Pasteur pipette 2 h before the bioassays, which were carried out in 24-well plates. Five neonates were transferred into each well, containing 10 ml water sample. The plates were covered and incubated at 20 °C in the dark. After 24 h incubation, the number of dead and immobilized neonates was recorded and the percent mortality calculated. The neonates were considered immobile if they remained settled at the bottom of the test container and did not resume swimming within the 15 s observation period. Tests were performed in quadruplicate and freshwater controls included in every test.

Results and discussion

Physicochemical characteristics of the wastewater samples

The physico-chemical qualities of the influent and effluent samples tested in this study on the three occasions are shown in

Table 1 Chemical composition of wastewater from two treatment plants at three sampling times

		Occasion 1				Occasion 2				Occasion 3			
		WWTP1		WWTP2		WWTP1		WWTP2		WWTP1		WWTP2	
Component	Guide[a]	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
PO_4 (mg L^{-1})	1	9.90	2.70	9.20	6.90	7.80	4.80	6.20	4.30	6.70	7.30	7.70	4.30
NH_4 (mg L^{-1})	1	54.0	18.0	50.0	4.80	42.0	23.0	28.0	4.90	42.0	24.0	29.0	4.90
$NO_3 + NO_2$ (mg L^{-1} N)	1.5	nd	0.33	nd	11	nd	<0.1	nd	14	nd	0.1	nd	14
As (mg L^{-1})	0.1	2.68	2.00	1.46	1.01	1.91	1.39	0.93	0.66	0.46	0.17	0.41	0.14
Pb (mg L^{-1})	0.1	3.72	3.57	3.70	3.55	3.59	3.53	3.59	0.70	3.54	3.53	3.51	3.51
Hg (mg L^{-1})	0.02	2.95	1.22	1.53	0.85	0.88	0.69	0.70	0.64	0.65	0.57	0.54	0.51
Cd (mg L^{-1})	0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BOD_5 (mg L^{-1})	5	317	35	360	45	112	17.0	240	56.0	143	20	243	19.0
COD (mg L^{-1})	30	284	97.0	820	81.0	456	115	871	62.0	797	196	416	81.0
pH	5.5–7.5	7.40	7.40	7.30	7.20	7.60	7.40	7.40	7.20	7.50	7.20	7.20	7.40

[a] Government Gazette (1984). Requirements for the purification of wastewater or effluent, Gazette number 9225 (nd = not detected; detection limits for heavy metals (in mg L^{-1}) are as follows: As (0.053), Cd (0.0027), Hg (0.061) and Pb (0.042)).

Table 1. The COD levels of the final treated effluent samples ranged between 97 and 196 mg L^{-1}, and 62 and 81 mg L^{-1} for WWTP1 and WWTP2, respectively, with a BOD level as high as 317 mg L^{-1} and 360 mg L^{-1} observed in the influent samples collected from WWTP1 and WWTP2, respectively. A significant reduction in BOD of the wastewater ranging from 85–100% and 77–100% was achieved by WWTP1 and WWTP2, respectively, with a maximum of 93% COD reduction observed for WWTP2. The BOD_5/COD ratio of the treated effluent falls within the recommended limit (<0.5), except on occasion two of the collection when the ratio was 0.9 for the treated effluent from WWTP2, thus indicating the high biodegradation of the wastewater during the treatment process.[19] The pH values ranged narrowly between 7.2 and 7.6 and 7.2 and 7.4 for the influent and effluent water samples. Ammonium concentration of the treated effluent ranged between 4.8 and 24 mg L^{-1}, values representing 1.75–10.42-fold reduction in the concentration during the treatment process. The nitrate + nitrite concentration range of <0.1 to 0.33 mg L^{-1} N obtained for the treated effluent from WWTP1 falls within the acceptable limit of 1.5 mg L^{-1}, while those of the final effluent from WWTP2 were generally above the limit on all occasions, with a concentration as high as 14 mg L^{-1} N detected. The heavy metal concentrations (mg L^{-1}) ranged between 0.14 and 2.00 for As, 0.70 and 3.57 for Pb and 0.51 and 1.22 for Hg, while cadmium was not detected in all the samples. Generally, effluents from WWTP1 contained a higher concentration of ammonia, arsenic and mercury, however, wastewater from WWTP2 has higher BOD level compared to that from WWTP1. According to the wastewater general limit values applicable to the discharge of wastewater into a water source as contained in the amended South African Water Service Act no. 54 of 1996 and Water Act no. 36 of 1998, the final effluents from these treatment plants meet the regulated guideline of 10 mg L^{-1} for phosphate and the pH range of 5.5–9.5.[20] However, except for WWTP2 on one occasion, they fail to comply with the recommended standard of 75 mg L^{-1} for COD. However, none of the samples meet the special standard stipulated as the requirements for the purification of wastewater or effluents in the Government Gazette (Gazette no. 9225, Regulation 991) as shown in Table 1. Non-compliance to some of the recommended limits has also been previously reported for some wastewater treatment plants in rural and semi-urban areas of South Africa.[21]

Toxicity monitoring of wastewater using bacterial biosensors and *Daphnia* toxicity assay

The toxicity effects of the influent and effluent wastewater samples from both WWTP1 and WWTP2 on three different collection times to both the bacterial biosensors and *D. magna* are shown in Fig. 1. As illustrated in the figure, variable responses, ranging from noninhibition to 100% toxic effects were observed for the test organisms. Overall, the influent samples from WWTP1 were found to be the most toxic to all the test species, with the toxicity effects ranging variously from 72–100%, 38–100% and 0–86% at different collection times (Fig. 1a). *Daphnia magna* was found to be the most sensitive for detecting the toxicity of the wastewater, compared to the two bacterial biosensors, with a mortality of 60–100% and 15–95% obtained for influent and effluent water samples, respectively. Using 50%

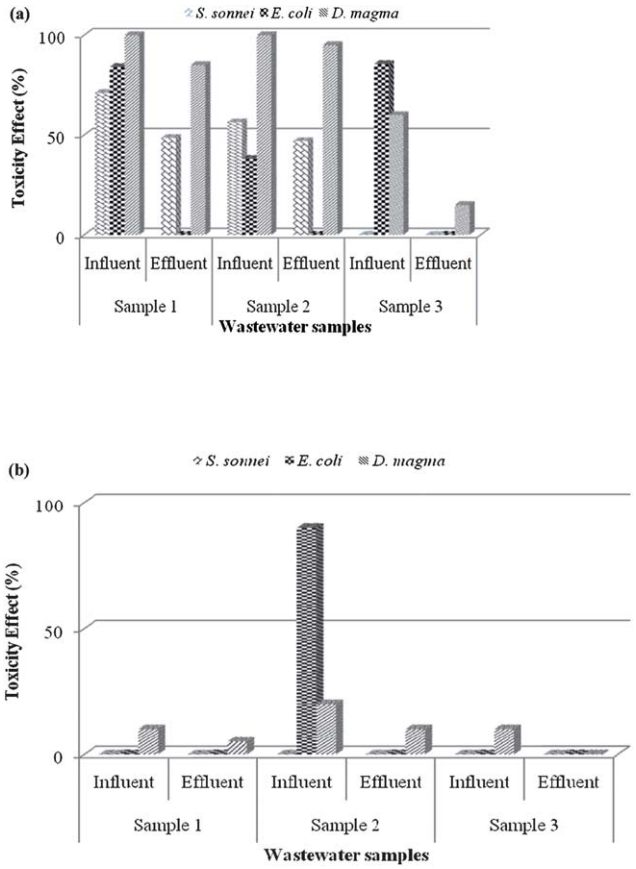

Fig. 1 Sensitivity of the bacterial biosensors and *D. magna* assay for detecting the toxicity of wastewater samples from (a) WWTP1 and (b) WWTP2 at different sampling times.

inhibition of the test species as the toxicity end point, both bacterial biosensors developed in this study compare favourably well with *D. magna* assay in indicating the toxicity level of the influent sample from the WWTP1 at the first collection time (Fig. 1a). Furthermore, like *D. magna*, the *S. sonnei* biosensor (sample 2) and *E. coli* biosensor (sample 3) were able to indicate the toxicity of the influent samples. Although, less sensitive the *S. sonnei* biosensor was able to respond negatively to the toxicity of the treated effluent samples, with a toxicity ranging from 0–49%, compared to a toxicity range of 15–95% obtained for *D. magna*. The wastewater from WWTP2 was not toxic to both the bacterial biosensors and *D. magna* with an induction of bioluminescence observed during exposure to the bacterial biosensors (Fig. 1b). This could be attributed to a high nutrient content or the low concentrations of toxic compounds in the effluent making the effluent act as a metabolic uncoupler, allowing the organism to counter the toxic challenge by increasing its metabolic rate.[22] However, the *E. coli* biosensor was found to be more sensitive to the toxicity of the influent wastewater from WWTP2, compared to *D. magna*, with up to 90% toxicity observed (Fig. 1b). In a test battery comprising several different organisms and *in vitro* test systems to determine the ecotoxicological effects of the additive butyl hydroxyanisole, the most sensitive assay was found to be the inhibition of *V. fischeri* emission, followed by the immobilization of the cladoceran *D. magna*.[23] *Daphnia magna* has also

been previously shown to be effective in assessing the toxicity of industrial textile dyes.[24]

The variation in the toxicity of wastewater from the different treatment plants could be attributed to the variability of the effluent composition as well as to the different sensitivity levels of each species tested.[8] The wastewater treatment plant designated as WWTP1 receives approximately 70% industrial wastes and 30% domestic wastes, whereas WWTP2 mostly receives domestic waste. Effluents from WWTP1 contained a higher concentration of ammonia, arsenic and mercury (Table 1). The biosensor response to the toxic challenge of these compounds commonly takes the form of a suppression of metabolic activity.[8] The observed higher sensitivity of *Daphnia* test compared to the bacterial biosensors is not uncommon as Hernando *et al.*[25] also reported the ability of *Daphnia* to detect pollutants at concentrations as low as ng L^{-1}. This study also shows that the *Daphnia* were able to detect the toxic effluent whereas the bacterial biosensors could not. Similarly, Barata *et al.*[26] and Soupilas *et al.*[5] showed that *Daphnia* responses were more sensitive than standardized acute assays when considering industrial contaminants.

Validation studies

To evaluate the predictive capability of the bacterial biosensors used in this study for estimating the amount of the toxicants present in wastewater, a concentration–toxic effect approach, in which their bioluminescence responses were evaluated for linearity over varying concentration ranges of the different heavy metals and inorganic contaminants present in wastewater, was used. A biphasic dose response in which low concentrations of the toxic compounds stimulated the bacterial luminescence and high concentrations exerted toxic effects was observed for both bacterial biosensors. The prevalence of hormetic dose responses to environmental toxins has been previously reviewed.[27] At certain concentration ranges, the bioluminescence response of the bacterial biosensors was found to be linearly correlated with increasing concentrations of both the heavy metals and inorganic compounds. The *S. sonnei* biosensor tends to be more accurate in estimating the concentration of heavy metals with r^2 values ranging between 0.954 and 0.988 for all the four heavy metals tested (Table 2), compared to the r^2 values of 0.824–0.944

Table 2 Correlations of bacterial bioluminescence response to the different concentrations of heavy metals and inorganic pollutants

Heavy metals/ inorganic compounds	Correlation coefficient (r^2)		Slope (% bioluminescence per mg L^{-1} concentration of the heavy metal)	
	E. coli	*S. sonnei*	*E. coli*	*S. sonnei*
Cadmium	0.824	0.988	1.599	63.00
Lead	0.935	0.964	5.103	1.395
Mercury	0.909	0.995	186.8	129.00
Arsenic	0.944	0.954	29.63	30.99
Ammonium	0.944	0.997	92.14	15 060
Nitrite	0.901	0.965	4616	837.5
Nitrate	0.981	0.874	98.32	2862
Phosphate	0.984	0.881	870.8	318.5

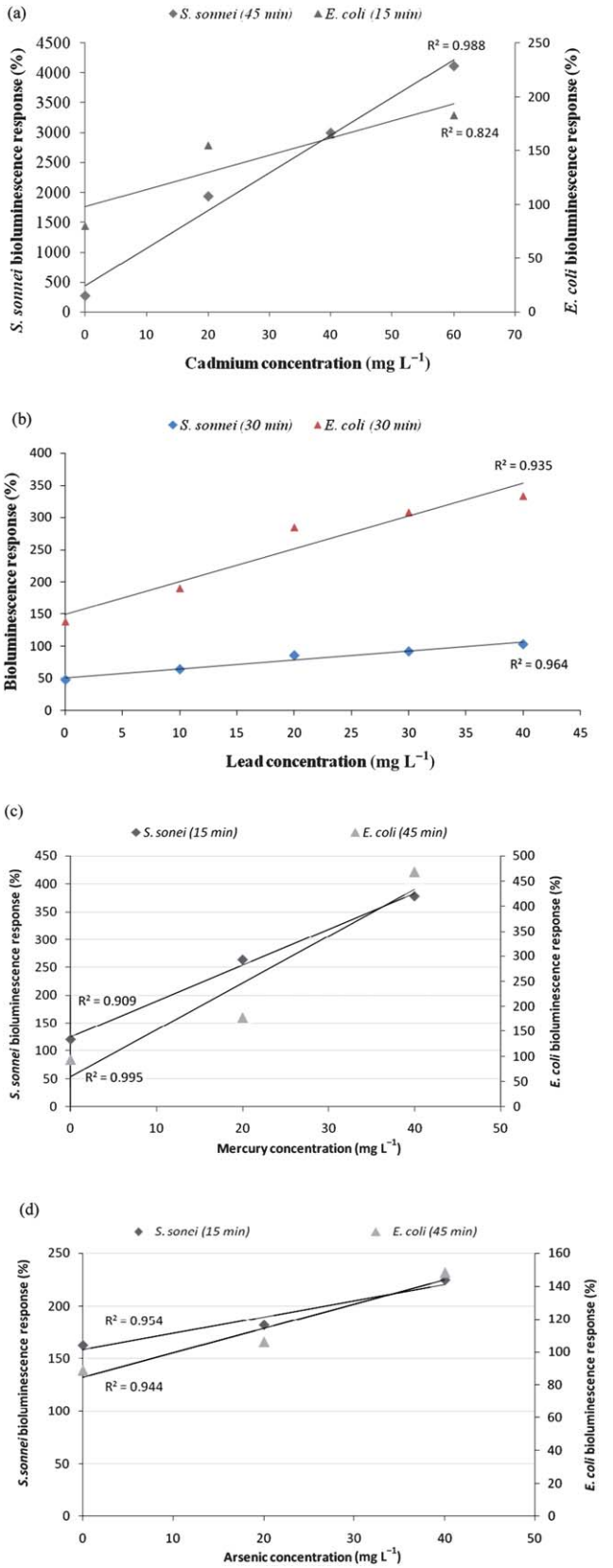

Fig. 2 Bioluminescence response of *S. sonnei* and *E. coli* biosensors to various concentrations of heavy metals (a) cadmium, (b) lead, (c) mercury and (d) arsenic.

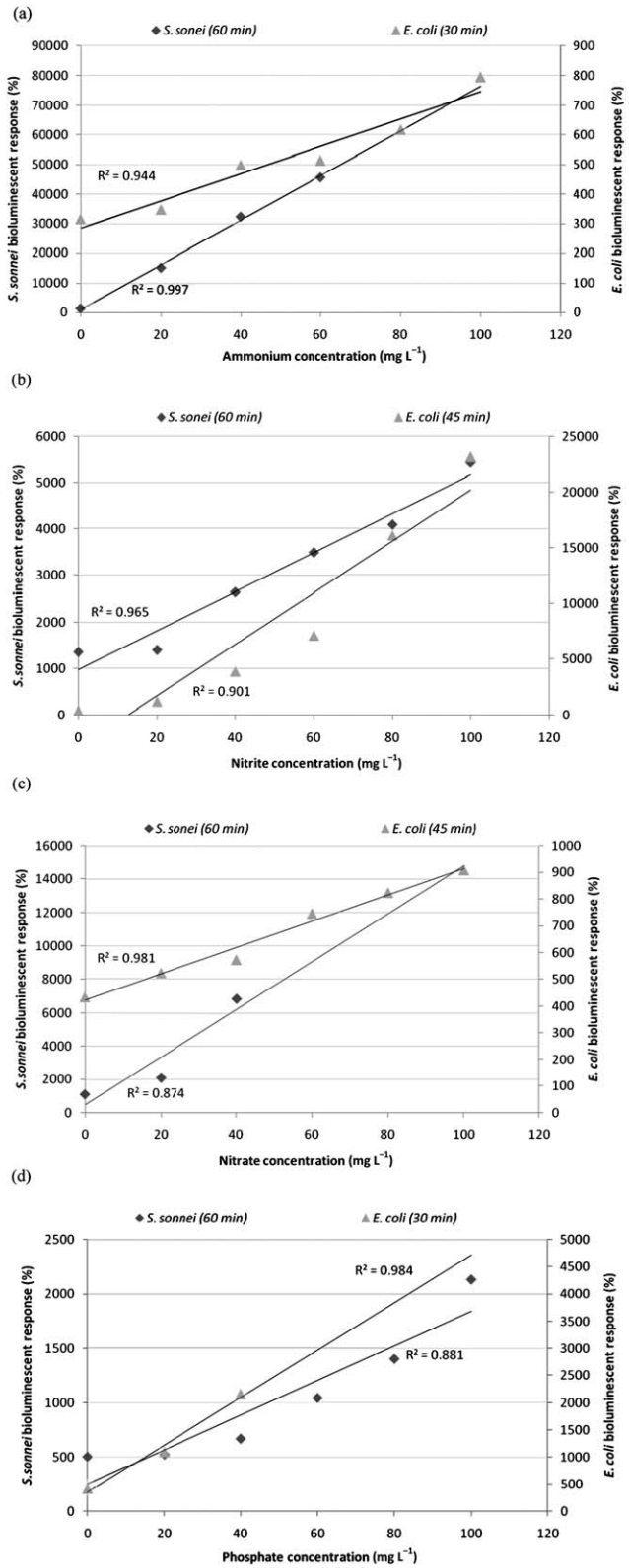

Fig. 3 Bioluminescence response of *S. sonnei* and *E. coli* biosensors to various concentrations of inorganic contaminants (a) ammonium, (b) nitrite, (c) nitrate and (d) phosphate.

obtained for the *E. coli* biosensor (Fig. 2). However, for the inorganic compounds (Fig. 3), *E. coli* was more sensitive to nitrate ($r^2 = 0.981$; slope = 98.32) and phosphate ($r^2 = 0.984$; slope = 870.8), while *S. sonnei* was found to be more sensitive to ammonium ($r^2 = 0.997$; slope = 15 060) and nitrite ($r^2 = 0.965$; slope = 837.5). Above the concentrations shown in the figures, a decrease in bioluminescence was observed, owing to the toxicity of the compounds to these bacterial biosensors at those concentrations. A variety of microbial bioluminescent biosensors have been constructed to detect metal contaminants in environmental samples.[28–31] For example, Lajoie *et al.*[28] developed Shock 1 (Shk1) bioreporter cells (*P. fluorescens* containing the *lux* genes) for toxicity monitoring in wastewater and obtained Zn EC_{50} bioluminescence values of approximately 7 and 32 mg L^{-1} for the influent wastewater and activated sludge mixed liquor, respectively, with Zn concentration as low as 1 mg L^{-1} being detected in the influent wastewater. A linear correlation was obtained up to 50 mg L^{-1} when the biosensor was tested between 0 and 200 mg L^{-1} concentration of Cd,[29] which is comparable to the observation in the current study, with a linear correlation obtained for both biosensors up to 60 mg L^{-1}. Similar to the findings of Ren and Frymier,[30] there was no general pattern for the relative sensitivity of the two biosensors to the heavy metals tested in this study, with the order of sensitivity As > Hg > Pb > Cd and Hg > Cd > Pb > As observed for *E. coli* and *S. sonnei* biosensors, respectively. However, lower concentrations (in μg L^{-1}) of $HgCl_2$ (0.003), $CdCl_2$ (1.8) and $PbNO_3$ (33) were detected by the "lights-on" sensor bacterial strains, constructed to express the luminescence encoding gene *luxCDABE* as a response to bioavailable heavy metals.[31] The greater sensitivity of bacterial biosensors to cadmium as observed in this study has been previously reported for other biosensor constructs.[32]

Conclusions

The bacterial sensors developed in this study were able to detect the toxicity of the wastewater tested. A linear correlation was also obtained between the luminescence response and pollutant concentrations, indicating the potential of these bacterial biosensors for rapid and effective monitoring of heavy metals and inorganic pollutants in water. Although there is a good correlation between the individual heavy metal and inorganic concentrations and bacterial bioluminescence response, necessary precautions should be taken when extrapolating results from a wastewater analysis, as wastewater represents a much more complex situation. Wastewater composition and constituent concentrations can be highly variable, and toxicity most likely results from a mixture of chemical compounds through interactive effects *i.e.* synergistic, antagonistic and additive between toxicants in a mixture. Consequently, the toxicity of a mixture cannot be inferred from the individual toxicity of the compounds. Most of the mixtures of pollutants have been observed to produce higher toxic effects by a factor between 3 and 10 times, indicating the increase in risk of these compounds in the environment when in combination.[25] This stresses the importance of complementing the chemical approach with the ecotoxicological approach, to maximize the environmental protection. Analysis of the effect of the mixtures of some of these compounds on the bioluminescence of the bacterial biosensors is

therefore a subject of on-going investigation in our laboratory. The sensitivity of these bacterial biosensors to the toxicants tested is promising for a simple, rapid, and cost effective detection and estimation of the concentrations of these compounds in contaminated water. However, the differences in sensitivity obtained for the different tested systems confirm the importance of the use of a battery of toxicity assays to provide a real assessment of the environmental risk of wastewater as no single organism responds appropriately to all toxicants.

References

1 S. Ren, *Environ. Int.*, 2004, **30**, 1151–1164.
2 C. Papadimitriou, G. Palaska, M. Lazaridou, P. Samaras and G. P. Sakellaropoulos, *Desalination*, 2007, **211**, 177–191.
3 S. Aguayo, M. J. Muñoz, A. de la Torre, J. Roset, E. de la Peña and M. Carballo, *Sci. Total Environ.*, 2004, **328**, 69–81.
4 W. Bourgeois, J. E. Burgess and R. M. Stuetz, *J. Chem. Technol. Biotechnol.*, 2001, **76**, 337–348.
5 A. Soupilas, C. A. Papadimitriou, P. Samaras, K. Gudulas and D. Petridis, *Desalination*, 2008, **224**, 261–270.
6 Y. Nomura, G. Chee and I. Karube, *Field Anal. Chem. Technol.*, 1998, **2**, 333–340.
7 E. Blübaum-Gronau and M. Hoffmann, *CNRS Res.*, 1997, **89**, 163–173.
8 S. Girotti, E. N. Ferri, M. G. Fumo and E. Maiolini, *Anal. Chim. Acta*, 2008, **608**, 2–29.
9 S. Audry, J. Schafer, G. Blanc and J. M. Jouanneau, *Environ. Pollut.*, 2004, **132**, 413–426.
10 T. A. Kurniawan, G. Y. S. Chan, W. H. Lo and S. Babel, *Chem. Eng. J.*, 2006, **118**, 83–98.
11 S. M. Taurianen, M. P. J. Virta and M. T. Karp, *Water Res.*, 2000, **34**, 2661–2666.
12 M. K. Habib, *Biosens. Bioelectron.*, 2007, **23**, 1–18.
13 J. Kumar, S. Kumar Jha and S. F. D'Souza, *Biosens. Bioelectron.*, 2007, **21**, 2100–2105.
14 A. A. J. Torriero, H. D. Piola, N. A. Martinez, N. V. Panimi, J. Raba and J. J. Silber, *Anal. Chim. Acta*, 2007, **71**, 1198–2004.
15 E. Akyilmaz, A. Erdoğan, R. Öztürk and İ. Yaşa, *Biosens. Bioelectron.*, 2007, **22**, 1055–1060.
16 A. O. Olaniran, R. M. Motebejane and B. Pillay, *J. Environ. Monit.*, 2008, **10**, 889–893.
17 J. Sambrook, E. F. Fritish and T. Manniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2nd edn, 1989.
18 DWAF, *Quality of Domestic Water Supplies. Sampling Guide 2*, Department of Water Affairs and Forestry, Department of Health and Water Research Commission, 1999.
19 Metcalf and Eddy, Inc., *Wastewater Engineering: Treatment, Disposal and Reuse*, McGraw-Hill, New York USA, 3rd edn, 1985.
20 S. Veenstra, G. J. Alaerts and M. Bijlsma, *Technology Selection: Water pollution Control*, E and FN Spon, London, 1997.
21 M. N. B. Momba, A. N. Osode and M. Sibewu, *Water SA*, 2006, **32**, 687–692.
22 M. Farre, O. Pasini, M. C. Alonso, M. Castillo and D. Barcelo, *Anal. Chim. Acta*, 2000, **426**, 155–165.
23 L. N. Santos, K. J. Smith, D. Davis and E. Delva, *Disinfection of Wastes in the New Millennium*, New Orleans, LA, United States, March 15–18, 2000, p. 171.
24 G. Dave and P. Aspegren, *Ecotoxicol. Environ. Saf.*, 2010, **73**, 1629–1632.
25 M. D. Hernando, A. R. Fernandez-Alba, R. Tauler and D. Bacelo, *Talanta*, 2005, **65**, 358–366.
26 C. Barata, P. Alanon, S. Gutierrez-Alonso, M. C. Riva, C. Fernndez and J. V. Tarazona, *Sci. Total Environ.*, 2008, **405**, 78–86.
27 E. J. Calabrese and R. Blain, *Toxicol. Appl. Pharmacol.*, 2005, **202**, 289–301.
28 C. A. Lajoie, S. C. Lin, H. Nguyen and C. J. Kelly, *J. Microbiol. Methods*, 2002, **50**, 273–282.
29 C. J. Kelly, N. Tumsaroj and C. A. Lajoie, *Water Res.*, 2004, **38**, 423–431.
30 S. Ren and P. D. Frymier, *Chemosphere*, 2005, **58**, 140–147.
31 A. Ivask, T. Rolova and A. Kahru, *BMC Biotechnol.*, 2009, **9**, 41–55.
32 D. Abd-El-Haleem, S. Ripp, C. Scott and G. Sayler, *J. Ind. Microbiol. Biotechnol.*, 2002, **29**, 233–237.