EXECUTIVE SUMMARY

General background and motivation of project

Pollution of water systems with chemicals and heavy metals poses a severe threat to human health and is of serious environmental concern. The detection of pollutants in the environment is time-consuming and expensive. Bacterial biosensors expressing the *lux* gene provide an alternative means of pollutant detection in the environment. This is made possible by the versatility of the metabolic and physicochemical characteristics of microorganisms. These biosensors offer a simple and convenient method to measure the acute toxicity of pollutants and are efficient tools in determining changes associated with complex chemical mixtures undergoing bioremediation. Microbial biosensors offer many advantages over chemical methods and other methods of ecotoxicity testing. Assays using microbial biosensors are rapid, sensitive, reproducible and cost-effective. These tests, based on the production of light by bacteria, reflect the effect of bio-available pollutants on the metabolic activity of the cells. The amount of light emitted is an indication of the presence of non-toxic or toxic substances which may induce or inhibit metabolism, respectively.

Original objectives of the study

i) To conduct a comparative study of existing luminescence-based assays in order to evaluate the cost-effectiveness as well as the relevance and appropriateness of these assays for the:
   - Determination of the bio-availability of pollutants in soil, groundwater and other water sources,
   - Detection of viable-but-non-culturable bacteria in soil, water supplies, groundwater and other water sources, and
   - Assessment of remediation and bioremediation potential;

It can be concluded from the literature survey that bacterial hosts incorporating the *luxCDABE* operon have proven to be a rapid and sensitive reporter system for the detection and monitoring of pollutants in environmental samples in developed countries. Therefore, the development of prokaryotic biosensors for local application would, in addition, also provide a cost-effective method of detecting environmental pollutants.

ii) To develop luminescence-based assays that would be appropriate, and cost-effective for the above applications;

iii) To compare the luminescence-based assay with standard water testing methods; and

iv) To transfer technology and develop local skills in luminescence-based assays.

Among the above-mentioned original objectives, (i) was covered in the Literature Review (Chapter Two), while (ii) - (iv) were addressed in the final objectives below.

Final objectives of the study

i) To construct prokaryotic biosensor systems using the *Vibrio fischeri luxCDABE* operon.
ii) To construct *Escherichia coli* DH5α, *Shigella sonnei*, *Shigella flexneri*, and Enteropathogenic *E. coli* biosensor systems using *lac*, *recA*, *fabA* and *uspA* promoters fused to the *V. fischeri luxCDABE* operon;
iii) To evaluate the sensitivity (minimum detection levels) of the biosensors' in the presence of laboratory standards of heavy metals and chemical pollutant.
iii) To compare the sensitivity of all the biosensor systems to existing acute toxicity tests, e.g., the *Daphnia* LC_{50} toxicity test and the *Vibrio fischeri*-based BioTox^{TM} kit.
v) To evaluate the sensitivity of the bacterial biosensors to heavy metals and chemical pollutants in wastewater effluent samples, from various Ethekwini Wastewater Treatment Works.

All the final objectives of this study were met. It should be pointed out that the final objectives represent a slight modification of the original objectives since these changes make them more relevant in terms of the outcomes of this research project. The approach to this study commenced with a literature review in Chapter Two. Thereafter, the study focused on achieving the final objectives which are reported in Chapters Three (i and ii), Four (iii and iv) and Five (v).

Methodology

Conventional methods and molecular methods were used. The conventional methods used were standard microbiological techniques, freeze-drying and measurement of bioluminescence. Molecular methods included the polymerase chain reaction (PCR) and standard molecular cloning techniques.

Summary of major findings and conclusions reached

- Several different bacterial biosensors with the ability to emit a readily detectable signal (light) in the presence of a wide range of environmental pollutants were developed. These included *luxCDABE*-containing *E. coli* DH5α, Enteropathogenic *E. coli*, *S. flexneri* and *S. sonnei* bacterial biosensor systems. The biosensors represent a fusion of bacterial bioluminescence (*lux*) genes, as a reporter, to selected bacterial gene promoters.
- The promoterless *V. fischeri luxCDABE* operon, responsible for light output, from pUCD607, was successfully integrated into the cloning vector pUC19, to yield the multi-copy 11 335 bp pLux plasmid.

- Plasmids pRecALux, pFabALux and pUspALux2 carrying the *lux* gene fused to stress inducible promoters were isolated from *E. coli* DPD2794, *E. coli* DPD2540 and *E. coli* DE135, respectively.

- *E. coli* DH5α, Enteropathogenic *E. coli, S. flexneri* and *S. sonnei* were successfully transformed with the plasmids pLux, pRecALux, pFabALux and pUspALux2 to create the bioluminescent biosensors: *E. coli* DH5α pLux, Enteropathogenic *E.coli* pLux, *S. flexneri* pLux, *E. coli* DH5α recA:lux, *E. coli* DH5α fabA:lux, *E. coli* DH5α uspA:lux and *S. sonnei* pLux.

- The prokaryotic biosensors were successfully freeze-dried using trehalose and Luria Bertani (LB) broth. Freeze-drying in trehalose consistently yielded uniform and stable freeze-dried products and only required 30 min of resuscitation, without agitation. It seemed that trehalose maintained the viability and biosensing activity of these biosensors, as seen by the high bioluminescence values after resuscitation in LB broth.

- *E. coli* DH5α pLux, Enteropathogenic *E.coli* pLux, *S. flexneri* pLux, and *S. sonnei* pLux exhibited a general inhibition in bioluminescence in the presence of heavy metals. However, the biosensors containing the stress inducible promoters viz., *E. coli* DH5α recA:lux, *E. coli* DH5α fabA:lux and *E. coli* DH5α uspA:lux exhibited a general induction in biolumescence at low concentrations of heavy metals.

- The different biosensors exhibited varying degrees of toxicity to the range of heavy metals tested. Cu (II) was most toxic to *E. coli* DH5α fabA: lux, *E. coli* DH5α uspA: lux, *S. flexneri* pLux, and Enteropathogenic *E.coli* pLux. Cr (VI) was most toxic to *E. coli* DH5α pLux while Zn (II) was most toxic to *E. coli* DH5α recA: lux.

- A comparison of the EC_{50} and LC_{50} with heavy metal compounds indicated that *S. sonnei* pLux was more sensitive than the commercially available *Vibrio fischeri*-based BioTox^{TM} kit and the traditional ecotoxicity test using *Daphnia*.

- The toxic effect of the different BTEX compounds varied among the biosensors. Xylene was most toxic and benzene least toxic to *E. coli* DH5α pLux, *E. coli* DH5α fabA: lux, *E. coli* DH5α uspA: lux and *S. sonnei* pLux. Xylene was also most toxic to *S. flexneri* pLux and Enteropathogenic *E.coli* pLux, while toluene was least toxic to these biosensors. In the case of *E. coli* DH5α recA: lux, ethylbenzene was most toxic and toluene least toxic.

- Effluent from the New Germany, Kwa-Mashu, Phoenix, Northern and Amanzimtoti wastewater treatment works induced bioluminescence in *E. coli* DH5α recA:lux, *E. coli*

DH5α fabA:lux and *E. coli* DH5α uspA:lux. However, the same wastewater effluent samples inhibited *E. coli* DH5α pLux, *S. flexneri* pLux, Enteropathogenic *E. coli* pLux and *S. sonnei* pLux.

- All biosensors were able to detect pollutants in the wastewater effluent samples at concentrations too low for detection with the *Daphnia* LC_{50} toxicity test.

- The data generated in this research demonstrates that the biosensors constructed in this study are potentially useful for the evaluation of environmental water samples and pollution management.

Review of the project in terms of the final objectives

All five of the final objectives of the study were achieved.

Recommendations for future research

Biosensors constructed in this study have the potential application to monitor environmental pollution. These whole cell biosensors hold a great deal of promise for continuous on-line monitoring of pollutant concentrations in environmental applications.

- The main application of these biosensor systems may be for the prescreening of environmental samples for toxic agents. Suspicious findings that indicate the presence of pollutants may then be verified using established physico-chemical methods utilised at environmental laboratories. This will reduce the costs of standard toxicity tests, e.g., *Daphnia* toxicity LC_{50} test. Therefore, efforts to design portable field devices that incorporate the biosensors constructed in this study will form the basis for future research.

- The ultimate aim will be to design a kit which can be used in the field by semi-skilled people with a basic microbiological background. The freeze-dried biosensors can be resuscitated on-site and the bioluminescent response of the wastewater samples measured directly using the portable 1254 - 001 LUMINOVA luminometer (Bio-Orbit Oy, Finland).

- Probably one of the greatest advantages of using these biosensors in toxicity evaluation of environmental pollution is that they can indicate the bioavailability of pollutants in a way that chemical analyses cannot. Continued improvement of these biosensors can meet the urgent need to not only quantify bioavailable pollutants, but also to perform *in situ* monitoring of biodegradation (Neilson et al., 1999). These biosensors have the

potential to offer a risk assessment strategy to predict the level to which a contaminated site may be bioremediated.

- The growing interest in employing whole-cell biosensors for the early detection of specific substance reinforces other potential uses apart from bioremediation and bioavailability assays. These applications include environmental hazard evaluations; prosecution and defence of chemical-related activities in environmental litigation; management of the discharge of municipal and industrial waste; and corporate industrial decisions on product development, manufacture and commercialisation so as to avert potential pollution. Therefore, the successful integration of the powerful applications of biosensor technology in pollution management may be one alternative to reverse the years of global environmental mistreatment.

Recommendations for technology transfer

- A hands-on workshop of 1-2 days for all stakeholders in the water, waste-water and health sectors.

- A simplified manual of this method and its applications should be published and distributed to all stakeholders.

- Development of a low-cost and user-friendly kit for on-site use.

Capacity-building and corrective action

WRC-funding of this project has contributed to both capacity-building and corrective action. As a HDI the former University of Durban-Westville catered largely for the needs of disadvantaged students. The research initiatives undertaken within the scope of this project have been in keeping with the University's Mission Statement as well as our Departmental Mission Statement, i.e. to develop and train scientists from previously disadvantaged communities and the establishment of a research culture at the University.

The following were achieved:

- Research culture:
  A vibrant and sustainable research culture was developed within the Department of Microbiology. The project facilitated the development and training of disadvantaged students and staff. Furthermore, technology was transferred from other institutions and research collaboration was fostered. This project has also contributed to increasing the critical mass of our Departmental research team.

- Students:
  Postgraduate student numbers have increased as a consequence of the implementation of the project. Twenty nine postgraduate students have successfully completed their studies within this project. Details of these students are included in Appendix Two.

- Improvements in primary, secondary and higher education:
  Through the Microbiology Students we have been able to make Microbiology accessible to schools. Pupils, Science teachers and Career Counselors are invited to the Department and exposed to various aspects of Microbiology (curriculum, entrance requirements, job opportunities and entrepreneurial opportunities.

- Community outreach:
  A research programme with relevance to the wider community has been established in Water Microbiology. This will now form one of the focus areas of the Department. Under-developed areas with poor sanitation and contaminated water supplies will be targeted with a view to improving the quality of life for disadvantaged sectors of the population.

Publications and conference proceedings

These are listed in Appendix Three.