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TECHNICAL BRIEF

DRINKING WATER

A suitable, on-line, real-time enzyme diagnostic system has been developed for the rapid detection and monitoring of sewage levels in drinking water.

Rapid Detection of Faecal Contamination

The threat of faecal contamination

Water intended for drinking purposes is under constant threat from a wide variety of pollutants in the environment. Inadequate sanitation frequently results in the channelling of untreated sewage and faecal material into freshwater catchment areas and riverine as well as marine systems. This increases the potential of collapse of the national drinking water supply and of outbreak of waterborne diseases such as cholera and cryptosporidiosis.

The challenge of routine testing

Tests for pathogens or, rather, indicator microorganisms (i.e. total and faecal coliforms, ***E. coli***, etc.) are currently in place to detect and quantify the presence of faecal contamination. However, these tests require laborious and time-consuming procedures and, consequently, are not favoured for routine use as rapid detection methods.

Rapid detection through enzymatic biosensors

Past research succeeded in establishing the feasibility of using a suitable biosensor system for the rapid enzymatic detection of indicators of faecal contamination in water. It was subsequently proposed that the most rapid and cost-effective way of building on this was to develop an on-line monitoring system through the use of a sequential flow injection analysis system with a spectro-photometric detector, either alone or in combination with an electrochemical detector.

Further research was consequently aimed at translating this concept into a working product, fit for use under a wide range of conditions such as varying ambient temperatures and the presence of potentially interfering substances, of chemical or biological origin, in the environment.

The end product of the research was envisaged to be a **suitable, on-line, real-time enzyme diagnostic system** that uses appropriate marker enzymes for the rapid detection and monitoring of sewage levels in drinking water.

Besides aiming to finalise the engineering of such a system, the research thus also focused on establishing the feasibility of using this biosensor for testing water in the presence of a wide range of chemical and biological substances, such as algal matter and high contents of salt, metal and phenolic compounds, in order to assess the potential for interference by these substances. The aim was also to compare the results of using this system with those of more established means of determining water contamination, including the use of commercial products currently available.

A final objective was to establish a resource base for the enzymology of sewage and industrial wastewater treatment processes. These aims were all successfully achieved, culminating in the development of a biosensor system covered by a South African patent.

Investigations in support of system development

The electrochemical detection of *Escherichia coli* β-D-glucuronidase (GUD) activity as a means of monitoring water pollution by faecal material was investigated using separate ***Moraxella***- and ***Pseudomonas putida***-modified glassy carbon electrodes. The ***Moraxella***-modified biosensor was 100 times more rapid and sensitive than the spectro-photometric detection of β-D-glucuronidase activity. The experimental limit of detection of the biosensor was 2 CFU.100 $m\ell^{-1}$ of polluted water sample within 20 minutes. The biosensor gave a linear response to commercial β-D-glucuronidase concentration between 0.2 ng and 2 µg $m\ell^{1}$.

In addition, a voltametric sensor prepared by the immobilisation of phthalocyanine metal complexes onto a glassy carbon electrode was developed for the detection of

β-D-galactosidase (GAL) of faecal origin in water. Electro-oxidation detection of chlorophenol red (CPR), a breakdown product of the chromogenic substrate chlorophenol red β-D-galactopyranoside (CPRG), was used as a measure of β-D-galactosidase activity.

Continuous multiple scans with the resultant reduction in fouling was possible, especially with a copper phthalocyanine-modified glassy carbon electrode. Although the sensor was sensitive to other phenolic compounds, these could be differentiated based on the potentials at which they occur.

The sensor was more sensitive in the acidic pH range and not significantly affected by temperature variations. The copper phthalocyanine metal complex-modified glassy carbon electrode could detect 1 CFU/100 mℓ in 15 minutes. A loss of 40% in sensitivity was, however, observed over a period of 30 days with the copper phthalocyanine-modified glassy carbon electrode.

Application potential of the biosensor system

The use of environmental samples for ***in situ*** GUD/GAL assays assisted in extending the application of the laboratory-developed protocols to the field. Electrochemical detection has subsequently been applied successfully to rivers in the Eastern Cape.

In conclusion, the investigations have shown that:

- Electrochemical detection of GUD activity using a ***Moraxella*** 1A modified GCE is rapid, cost-effective, sensitive and feasible for on-line and real-time monitoring of faecal pollution in water;
- Electrochemical detection of GAL activity using a CPRG-based biosensor, especially with the use of a copper phthalocyanine-modified glassy carbon electrode is also rapid, cost-effective, sensitive and feasible for on-line and real-time monitoring of faecal pollution in water and is, in fact, probably the most viable as it does not require live cells for the proper functioning of the biosensor;
- Both biosensors (***E. coli*** GUD and CuPc-modified GAL biosensors) are not susceptible to algal interference. This can be attributed to the multiple enzymes involved in signal generation and the differences in the optimum pH of the algal GUD and ***Moraxella*** enzymes; and
- Both GUD and GAL can, therefore, be used for the development of an alternative method for rapid on-line and real-time monitoring of microbial water quality in faecal-contaminated water.

The cost of a new, market-ready technology can often be prohibitive to poorer communities. In this instance, the difficulty is further compounded by the need to combine potentially antagonistic attributes, such as simplicity, cost-effectiveness and automation, in the biosensor design.

In the light of cost considerations, the ***E. coli*** biosensor resulting from the described research can be manufactured in two forms to meet both low-end (poor rural communities) and high-end (local authorities and water suppliers) markets. For the low-end market, single-use disposable electrodes and portable potentiostats could be produced, while for the high-end market, a more automated, continuous, on-line instrument could be designed, based on a sequential flow injection analysis (SFIA) system.

Possible future developments

While this study has shown that GUD/GAL could be used for on-line electrochemical detection of faecal contamination in polluted water, it has also enabled other perspectives, relating particularly to needs for further research, to be gained.

Some of these needs, for example, relate to:

- Automation of the proposed SFIA;
- Investigation of the temporal distribution of GUD/GAL in water bodies; and
- Extension of this biosensor application to other organisms and toxins.

Further reading:

*On-line Real-time Enzymatic Biosensor System for the Rapid Detection of Faecal Contamination of Water Intended for Drinking Purposes (***Report No: 1603/1/08***).*
To order this report contact Publications at Tel: (012) 330-0340; Fax: (012) 331-2565 or E-mail: orders@wrc.org.za; Web: www.wrc.org.za