

Rapid Toxicity Assessment

A new method based on quantifying mediated microbial respiration at a microelectrode

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Introduction

Environmental pollution is of wide concern and subject to prescriptive legislation (Yeates *et al.*, 1994). Enforcement of such legislation necessitates accurate, reliable and cost effective monitoring techniques. Conventional analytical techniques offer high precision but are disadvantaged by the high cost of specialised equipment, the need for trained personnel and are not amenable to rapid on-site analysis. Hence there is a general need to develop simple, inexpensive, rapid and sensitive tests for use by the regulatory agencies for environmental testing (Qureshi *et al.*, 1984). Biosensors are an analytical tool which have the potential to fulfill the analytical needs of regulatory agencies.

Lincoln Technology has developed a rapid biosensor-based assay called MICREDOX™. While MICREDOX™ was originally developed for BOD monitoring, here we report how this rapid assay has been extended to ascertain the impact of toxic chemicals on biological materials resident in the environment.

The MICREDOX™ assay accelerates the microbial oxidation of organic substrates by using a redox mediator (ferricyanide ion), instead of oxygen, as the terminal electron acceptor. This substitution increases the rate of substrate oxidation, partly because the mediator is highly soluble, but principally because a surplus of mediator allows a higher microbial concentration to be used, facilitating more substrate oxidation without depleting the electron acceptor. Substrate conversions (60.5%), equivalent to those obtained in 5 days with the BOD₅ method, are achieved in 1 hour or less (Pasco *et al.*, 2000). The microbially reduced mediator accumulates as a product registering the amount of bioconversion (Fig. 1). Electro-analytical techniques, either bulk electrolysis or limiting-current microelectrode amperometry, are used to measure the quantity of reduced mediator, giving a direct measure of the microbially catalysed substrate oxidation.

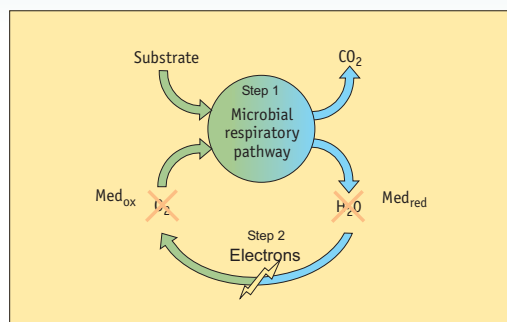


Fig. 1 MICREDOX™: 2-step

When MICREDOX™ is operating in toxicity mode, the charge (required to re-oxidise the reduced mediator) produced by healthy cells is compared to the charge produced by cells that have been subjected to a fixed level of toxin. For example, toxins present in high enough concentrations will compromise the respiration of the organisms, resulting in a reduction in the amount of reduced mediator and thus a smaller charge will be detected. (Pasco *et al.*, 2001).

Bulk electrolysis, although a robust and absolute method for measuring the amount of reduced mediator, is impaired by being a destructive and time consuming technique. Limiting-current microelectrode amperometry is an alternative method, which is both rapid and non-destructive, for determining the concentration of reduced mediator. At microelectrodes, the diffusion of electroactive species to and from the electrode is unimpeded, unlike macro electrodes, and the current comes to steady state within seconds of an appropriate working potential being applied. This behaviour of microelectrodes is characterised by the absence of peaks in a cyclic voltammogram; because in the diffusion-limiting region the current is independent of both time and potential according to the modified Cottrell equation. Limiting-current microelectrode amperometry, because the sampling is non-destructive and faster than bulk electrolysis, provides opportunity for gathering time-series data and possibly continuous monitoring.

Aim

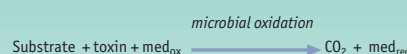
To demonstrate that the MICREDOX™ technology can rapidly assess the toxicity of compounds using 3, 5 - dichlorophenol (DCP), copper (Cu (II)), chromium (Cr (VI)), and arsenate (As (V)).

Method

MICREDOX™ Rapid Toxicity Assay is a two-step process:

Step 1. Incubation

Microorganisms are incubated for a fixed time with known amounts of substrate (glucose/glutamic acid mixture), toxin and redox mediator.

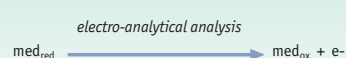


Incubation volumes per assay

| Cells | Mediator | Substrate & Toxin |
|----------------------------------|------------------|--|
| 4.00 ml (OD ₆₀₀ = 25) | 4.33 ml (0.25 M) | 11.83 ml spiked with known amount of toxin |
| 33.3mg | 1082 μmole | 10.82 μmole |

Step 2. Signal detection

Bulk electrolysis and limiting current microelectrode amperometry were used for electro-analytical analysis of the quantity of microbially reduced mediator.



The MICREDOX™ Rapid Toxicity Assay was used to measure the impact of DCP on the bacteria *Escherichia coli*, *Pseudomonas putida* and *Bacillus subtilis*. The toxic impact of Cu (II), Cr (VI) and As (V), the metals widely used in antifungal wood preservatives, were also monitored and were carried out as single, binary and ternary mixtures. The concentration of toxin required to reduce microbial respiration by 50% (EC₅₀) was established for all compounds, singly and in mixtures.

Conclusions

- The MICREDOX™ system can assess the toxicity of compounds in 60 min or less (Fig. 3).
- The MICREDOX™ system responds in a dose dependent manner and the EC₅₀ values obtained relate well to values reported in the literature.
- The MICREDOX™ EC₅₀ values for DCP are constant across the three microbial species tested (Table 1).
- The binary and ternary mixtures exhibited antagonistic behaviour.
- Limiting-current microelectrode is an equivalent detection technique to bulk electrolysis. Microelectrode amperometry correlates in a strong positive linear manner to bulk electrolysis ($r^2 = 0.9716$) (Fig. 2).
- The collection of time course data provides dynamic information about the evolution of the toxic response throughout the MICREDOX™ assay. It gives the flexibility to look at both short and long term toxic effects (Fig. 3).
- The technology and materials are inexpensive, readily available and amenable to miniaturisation.
- This is a relative technique that can report either the increase in respiration derived from the presence of substrate or total respiration (incremental and endogenous).

Results

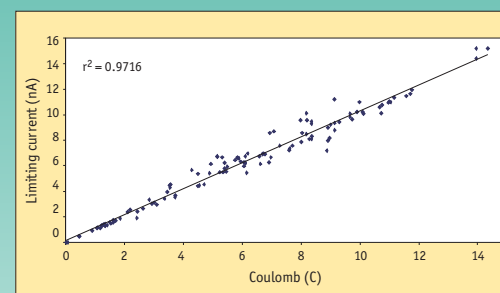


Fig. 2 Correlation between bulk electrolysis results and limiting-current microelectrode amperometry for quantifying the reduced mediator.

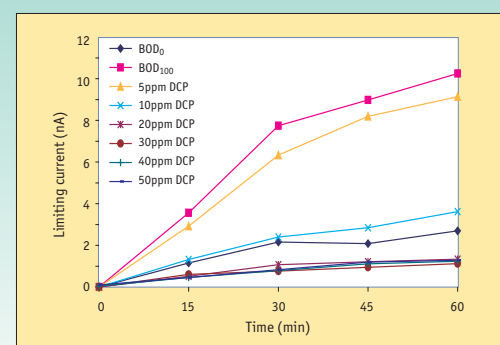


Fig. 3 Temporal variation in limiting currents. *B. subtilis* spiked with DCP

Table 1 EC₅₀ values obtained from 60-min incubations with MICREDOX™

| Toxin and Microorganism | EC ₅₀ (ppm) |
|--|---|
| DCP, <i>E. coli</i> | 7.0 |
| DCP, <i>Ps. putida</i> | 8.5 |
| DCP, <i>B. subtilis</i> | 7.5 |
| Cu (II), <i>B. subtilis</i> | 61 |
| Cr (VI), <i>B. subtilis</i> | 68 |
| As (V), <i>B. subtilis</i> | 154 |
| Cu (II) fixed at 70ppm/ Cr (VI) binary, <i>B. subtilis</i> | 161 (Cr (VI)) |
| Cu (II) fixed at 70ppm/ Cr (VI) fixed at 35ppm/ As (V) ternary, <i>B. subtilis</i> | not obtained as 50% inhibition was outside the range tested |

Acknowledgement

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