

# Application of Sterikon® bioindicators for the determination of bactericide concentrations

TE Cloete,\* E Da Silva and VS Brözel

Environmental Biotechnology Laboratory, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

## Abstract

Biofouling in industrial water systems is normally prevented by the use of bactericides. However, bactericide programmes often fail owing to the lack of suitable techniques for determining the *in situ* bactericide concentration and this usually results in either inadequate or excessive bactericide concentrations. In this study, the Sterikon® bioindicator was evaluated for determining the minimum inhibitory concentrations of 5 industrial bactericides (dichlorophen, sulphone, thiocarbamate, isothiazolone and a quaternary ammonium compound) for the monitoring of the concentrations of these compounds in industrial water systems. The results indicated that the Sterikon® bioindicator can be used for the determination of bactericide concentrations.

## Introduction

Water cooling systems contain a variety of bacteria which colonise surfaces. This leads to biofilm formation and subsequent biofouling and microbially induced corrosion (Cloete et al., 1992). The efficacy of bactericide programmes for biofouling control in industrial water systems relies not only on the spectrum of antibacterial activity of the bactericide, but also on the available concentration (Cloete et al., 1989; Brözel and Cloete, 1991a). In many cases the correct available concentration is not attained due to a lack of knowledge on the size of the system or the difficulty to determine the residual concentration of the bactericide. In recirculating water systems, bactericide concentrations decrease after addition due to system blow-down and interaction with bacteria (Cloete et al., 1992; Warner, 1985). Normal practice would be to add bactericide periodically to maintain the required concentration. It is emphasised that the concentration of a bactericide is not linearly related to its activity; a concentration exponent is involved in the relationship (Hugo and Denyer, 1987). In the case of most bactericides, a small decrease in concentration will result in a large decrease in activity. For bactericide programmes to be effective one would ideally want sufficient available bactericide and an adequate exposure time. This would prevent the depletion of the bactericide to sublethal concentrations and minimise the risk of bacteria becoming resistant to a specific product (Brozel and Cloete, 1991b). Due to the difficulty in determining available *in situ* bactericide concentration, rates of depletion due to inactivation are unknown and this has led to the mismanagement and failure of many biofouling control programmes.

The concentrations of non-oxidising bactericides can be determined by conventional analytical means. Most of these involve extraction followed by instrumental analysis. These techniques are sophisticated and cumbersome, and too lengthy and expensive for routine use. Rapid convenient tests are available for some oxidising bactericides, e.g. Merckoquant® peroxide for hydrogen peroxide and Merckoquant® chlorine for chlorine determinations.

In practice most bactericides react with substances contained in the water, decreasing the available concentration. Furthermore, even if the residual concentration could be determined accurately, it would not reflect the antimicrobial activity of the product. Techniques for the determination of antimicrobial activity of bactericides are available (Payne, 1988; Cloete et al., 1989; Hill et al., 1989). Results obtained using laboratory methods of bactericide evaluation cannot generally be related to the practical situation (Payne, 1989; Cloete et al., 1989; Brozel and Cloete, 1992). Nevertheless, these tests do provide useful information during the development of bactericides.

A more serious problem is the lack of suitable techniques for the *in situ* determination of available bactericide concentrations. In this regard one bioindicator has been developed (Hill et al., 1989). Bioindicators are considered to be biological preparations that usually contain spores of a single bacterial strain with a known susceptibility towards an antimicrobial agent. Sterikon® is used as a bioindicator in heat sterilisation. It is a glass vial containing spores of the apathogenic *Bacillus stearothermophilus* ATCC 7953 suspended in a broth containing glucose and a pH indicator. After heat exposure the vial is incubated at 45°C and viable spores germinate, produce acid and render the indicator yellow. A yellow vial is indicative of insufficient heat treatment. Sterikon® has not been evaluated before for the determination of bactericide concentrations. The objective of this study was, therefore, to determine whether Sterikon® could be used as a bioindicator for the *in situ* determination of available bactericide concentrations.

## Materials and methods

### Bactericides used

- Dichlorophen (2,2'-methylenebis(4-chlorophenol), 40% (m/v) solution) (BDH Chemicals)
- Sulphone (bis-trichloro-methyl sulphone, commercial solution) (Chemserve Systems)
- Quaternary ammonium compound (tetradecylbenzyl-dimethyl-ammonium chloride syrup) (Merck)
- Thiocarbamate (sodium dimethyldithiocarbamate) (Fluka)
- Isothiazolone (10,1% 5-chloro-2-methyl-4-isothiazolin-3-one and 3,8% 2-methyl-4-isothiazolin-3-one) (Thor chemicals).

\*To whom all correspondence should be addressed.

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