Short communication

Application of Sterikon® bioindicators for the determination of bactericide concentrations

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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 (Brözel 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.

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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; Brözel 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 (tetradecylbenzyldimethylammonium 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).

TABLE 1
MIC FOR THE ISOTHIAZOLONE BACTERICIDE USING STERIKON®

Bactericide concentration ($\mu l \cdot l^{-1}$)	Incubation time (h)					
	8	18	24	28	36	48
Control	-	+++	+++	+++	+++	+++
5	-	+++	+++	+++	+++	+++
10	-	- - +	+++	+++	+++	+++
15	-	- - -	- ++	- ++	+++	+++
20	-	- - -	- - -	- - +	- - +	+++
30	-	- - -	- - -	- - -	- - -	- - -
40	-	- - -	- - -	- - -	- - -	- - -
50	-	- - -	- - -	- - -	- - -	- - -

\+ = growth (yellow)
\- = no growth (purple)
The standard MIC was taken as 30 $\mu l \cdot l^{-1}$ due to the lack of growth after 48 h incubation.

TABLE 2
MIC FOR THE THIOCARBAMATE BACTERICIDE USING STERIKON®

Bactericide concentration ($mg \cdot l^{-1}$)	Incubation time (h)					
	8	18	24	28	36	48
Control	-	+++	+++	+++	+++	+++
5	-	- - +	+++	+++	+++	+++
10	-	- - -	- - -	- - +	+++	+++
15	-	- - -	- - -	- - +	+++	+++
20	-	- - -	- - -	- - -	+++	+++
30	-	- - -	- - -	- - -	- - -	- - +
40	-	- - -	- - -	- - -	- - -	- - -
50	-	- - -	- - -	- - -	- - -	- - -

\+ = growth (yellow)
\- = no growth (purple)
The standard MIC was taken as 30 $mg \cdot l^{-1}$ due to the lack of growth after 48 h incubation.

TABLE 3
MIC FOR THE SULPHONE BACTERICIDE USING STERIKON®

Bactericide concentration ($\mu l \cdot l^{-1}$)	Incubation time (h)					
	8	18	24	28	36	48
Control	-	+++	+++	+++	+++	+++
1	-	+++	+++	+++	+++	+++
2	-	- - -	- - -	- - -	- - -	+++
3	-	- - -	- - -	- - -	- - -	- - -
4	-	- - -	- - -	- - -	- - -	- - -
5	-	- - -	- - -	- - -	- - -	- - -
10	-	- - -	- - -	- - -	- - -	- - -
20	-	- - -	- - -	- - -	- - -	- - -
30	-	- - -	- - -	- - -	- - -	- - -
40	-	- - -	- - -	- - -	- - -	- - -
50	-	- - -	- - -	- - -	- - -	- - -

\+ = growth (yellow)
\- = no growth (purple)
The standard MIC was taken as 3 $\mu l \cdot l^{-1}$ due to the lack of growth after 48 h incubation.

TABLE 4
MIC FOR THE DICHLOROPHEN BACTERICIDE USING STERIKON®

Bactericide concentration ($\mu l \cdot l^{-1}$)	Incubation time (h)					
	8	18	24	28	36	48
Control	-	+++	+++	+++	+++	+++
0,1	-	+++	+++	+++	+++	+++
0,2	-	+++	+++	+++	+++	+++
0,3	-	+++	+++	+++	+++	+++
0,4	-	+++	+++	+++	+++	+++
0,5	-	+++	+++	+++	+++	+++
0,6	-	- - -	- - -	- - -	- - -	- ++
0,7	-	- - -	- - -	- - -	- - -	- - +
0,8	-	- - -	- - -	- - -	- - -	- - -

\+ = growth (yellow)
\- = no growth (purple)
The standard MIC was taken as 0,8 $\mu l \cdot l^{-1}$ due to the lack of growth after 48 h incubation.

Standardisation of bactericide concentrations

A range of concentrations of each bactericide was added directly to the ampoules. These were then sealed and incubated at 45°C for 24 h. All determinations were performed in triplicate. A clear violet colour after 24 h was taken as sufficient bactericide to inhibit spore germination, or alternatively, to kill the spores. Yellow ampoules were a sign of acid production due to germination and growth, indicating insufficient bactericide.

Interaction of bactericides with Sterikon®

The possibility existed that the bactericides used in this study could react with the indicator, or with other components in the ampoule, bringing about a colour change not due to bacterial acid production. Various bactericide concentrations were added to sterile aqueous solutions of the indicator bromocresol purple, and to Sterikon® and incubated at 25°C for 24 h. Incubation was at 25°C since spores of *B. stearothermophilus* germinate only at *ca.* 45°C. The metabolic activity of the bacteria was thus prevented without tampering with the contents of the Sterikon® vial. No colour changes to yellow were recorded, indicating that none of the bactericides evaluated interacted with the indicator to produce false positives.

TABLE 5
MIC FOR THE QUATERNARY AMMONIUM BACTERICIDE USING STERIKON®

Bactericide concentration	Incubation Time (h)					
($\mu l \cdot l^{-1}$)	8	18	24	28	36	48
Control	-	+++	+++	+++	+++	+++
0,1	-	+++	+++	+++	+++	+++
0,2	-	+++	+++	+++	+++	+++
0,3	-	- - -	- - -	- - -	+++	+++
0,4	-	- - -	- - -	- - -	- - -	+++
0,5	-	- - -	- - -	- - -	- - -	- - -

\+ = growth (yellow)
\- = no growth (purple)
The standard MIC was taken as 0,5 $\mu l \cdot l^{-1}$ due to the lack of growth after 48 h incubation.

Results

The results are given in Tables 1 to 5. To use Sterikon® as a method for determining unknown bactericide concentrations, it was necessary to determine the minimum concentration of each bactericide required to inhibit outgrowth of the spores. Note that this minimum inhibitory concentration (MIC) should not be interpreted as the efficacy of a product in general as it is specific for spores of *B. stearothermophilus*. The results for isothiazolone indicated that after 18 h the MIC was 15 $\mu l \cdot l^{-1}$, after 24 h the MIC was 20 $\mu l \cdot l^{-1}$ and after 28 h the MIC was 30 $\mu l \cdot l^{-1}$ (Table 1). No growth occurred at the latter concentration during the 48 h period of incubation and therefore this concentration (30 $\mu l \cdot l^{-1}$) was taken as the standard MIC for this product.

The MIC of thiocarbamate after 18 h incubation was 10 $mg \cdot l^{-1}$, after 24 h incubation 10 $mg \cdot l^{-1}$, after 28 h incubation 20 $mg \cdot l^{-1}$, and after 36 h incubation the MIC was 30 $mg \cdot l^{-1}$ (Table 2). No further growth was detected at the latter concentration after a 48 h period of incubation. Therefore, 30 $mg \cdot l^{-1}$ was taken as the MIC for the thiocarbamate bactericide. The results of the sulphone bactericide indicated that 3 $\mu l \cdot l^{-1}$ was sufficient to prevent growth (Table 3). No growth occurred after 48 h incubation at 3 $\mu l \cdot l^{-1}$; therefore this was taken as the standard MIC for the sulphone bactericide used. For dichlorophen the MIC after 18 h was 0,6 $\mu l \cdot l^{-1}$ (Table 4). After 48 h it was 0,8 $\mu l \cdot l^{-1}$. This indicated that 0,8 $\mu l \cdot l^{-1}$ was the standard MIC of dichlorophen in Sterikon®. The standard MIC for the quaternary ammonium compound used was 0,3 $\mu l \cdot l^{-1}$ after 18 h (Table 5). After 36 h it was 0,4 $\mu l \cdot l^{-1}$, and after 48 h it was 0,5 $\mu l \cdot l^{-1}$. Therefore, the standard MIC of the quaternary ammonium compound used was taken as 0,5 $\mu l \cdot l^{-1}$.

It is not surprising that the MIC value varied among the bactericides evaluated, as a given test organism would be more susceptible to certain bactericides than to others (Brözel and Cloete, 1991a). This is, however, not important in terms of using Sterikon® as a method of bactericide concentration determination. What is important is the fact that the MIC determined by Sterikon® should always be the same for the same bactericide.

Discussion

The results in this study indicate that the Sterikon® bioindicator can be used successfully for the determination of the concentration of bactericides in water samples. Since *B. stearothermophilus* spores germinate only at temperatures above 45°C, the Sterikon® ampoule must be incubated at this temperature. The bacterial flora of the water samples tested will, therefore, not interfere with the determination, as these bacteria are mostly mesophilic. They cannot grow at 45°C, or only at a very slow rate, and will not cause a colour-change to indicate an incorrect bactericide concentration. Water samples can, therefore, be used directly and do not have to be filter-sterilised as would be the case if the indicator organism itself were a mesophile.

This procedure was evaluated in the laboratory situation by giving samples of water containing an unknown bactericide concentration to laboratory technicians. They were able to determine the bactericide concentration correctly.

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