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74

RAPID METHOD FOR DETERMINING THE SENSITIVITY OF SULPHATE-REDUCING BACTERIA TO BIOCIDES

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ABSTRACT - The Minimal Inhibitory Concentration (MIC) of a biocide is usually determined by adding it in various concentrations to a suitable growth medium which is then inoculated with the test bacterium. These cultures are then observed for up to 28 days for growth of the organism as shown by its production of black ferrous sulphide.
A method has been developed whereby the MIC of a biocide can be found within 4 days. Instead of noting ferrous sulphide production, this new method measures changes in : 1) optical density and 2) sulphate reduction of a logaritmic culture of the test strain exposed to the biocide.

Key words: Minimal Inhibitory Concentration, sulphate-reducing bacteria, glutaraldehyde.

RÉSUMÉ - La concentration minimale inhibitrice (MIC) d'un biocide est habituellement déterminée par addition du biocide à différentes concentrations dans un milieu de croissance approprié qui est alors inoculé par la bactérie testée. Ces cultures sont ensuite suivies pendant un maximum de 28 jours pour mettre en évidence la production de sulfide ferreux noir. Une méthode permettant de déterminer le MIC d'un biocide en 4 jours a été mise au point. Cette méthode n'étudie pas la production de sulfide ferreux mais mesure les variation de 1) la densité optique et 2) de la réduction des sulfates dans une culture en phase logarithmique de croissance de la souche testée, exposée à l'action du biocide.

Mots clés: Concentration Minimale Inhibitrice, bactérie sulfato-réductrice, glutaraldéhyde.

INTRODUCTION

One of the simplest ways to measure the effect of a biocide on an organism is by determining the Minimal Inhibitory Concentration (MIC) which just prevents growth in a suitable medium. The antibacterial agent is serially diluted in the medium and a standardised inoculum of the test strain added. After incubation for a predetermined period, at the optimum temperature for growth, the cultures are examined and the MIC for the biocide noted. This bacteriostatic test is only a measure of bacterial inhibition and gives no information on the lethal effect of the agent under test. The bactericidal effect of a biocide can be found by a time-kill test. Here, a culture of the test organism is exposed to various concentrations of the biocide and the number of viable cells present at timed intervals estimated.

API biocide tests:

The recommended American Petroleum Institute (API) method for testing the efficacy of biocides against sulphate-reducing bacteria (SRB) is an MIC determination which requires an incubation of inoculated bottles for 28 days (Anon, 1975). The API also

recommend time-kill tests of SRB contaminated injection water which again take ca 28 days before a result is obtained.

Principle of the new method:

In an attempt to reduce the delay that is inherent in the API testing methods we have developed a simple and rapid technique for studying the effect of a biocide on SRB by measuring the inhibition of:
1) culture absorbance at 580 nm
2) sulphate reduction

- in a logarithmic culture of the test SRB strain.

On average only 4 days are required to complete the test. Although rapid radiorespirometric methods for biocide testing have been described recently (eg. Hardy and Syrett, 1983), such techniques require relatively sophisticated and expensive equipment and have attendant safety problems. The method described below is simple, rapid and inexpensive.

MATERIALS AND METHODS

The low iron medium (C) of Postgate (1984) is used supplemented with 0,1 g/l ascorbate and thioglycollate as reducing agents and 0.0002 % w/v resazurin as a redox indicator. This is dispensed in 9.5 ml amounts into a series of 20 ml capacity test tubes and autoclaved at 121°C until reduced. The tubes are cooled under a stream of oxygen-free nitrogen gas, sealed with butyl rubber stoppers and autoclaved with the tops clamped down for 15 mn at 121°C. Inoculation of the tubes is by injection of 0.2 ml of the test strain (30 hours at 30°C in medium C). SRB growth is followed by inserting the tubes into a colorimeter and measuring the increase in absorbance at 580 nm. In the early logarithmic phase of growth (A_{580} ca 0.15) dilutions of the biocide are injected into 4 tubes of the same A_{580} and the contents mixed. Control tubes are inoculated with sterile distilled water.

On biocide addition, and after, 3, 6, and 9 hours incubation the following parameters are determined:

1) absorbance at 580 nm
2) residual sulphate in the medium (eg ; $BaCl_2$ - EDTA titration)

On biocide addition and after 9 hours incubation:

3) the numbers of viable SRB are also determined as described by Postgate (1984).

RESULTS AND DISCUSSION

Figure 1 shows results typical of those obtained by this method. Alkaline glutaraldehyde, a 5-carbon dialdehyde, was tested against *Desulfovibrio desulfuricans* NCIB 8400. Biocide increments of 50 ppm were used as this interval is recommended for biocides with potential off-shore applications (Crouch, 1983). Inhibition of SRB growth occurred at a concentration of 50 ppm (Fig 1b).The effect of a biocide on a cell is normally dependant on its concentration and it can be seen from the slight increase and levelling off of A_{580} at 50 ppm that glutaraldehyde is bacteriostatic at this concentration. However, at 100 ppm the decrease in A_{580} reflects a bactericidal action with cell lysis. This preliminary observation is confirmed by the counts for viable SRB after 9 hours incubation.

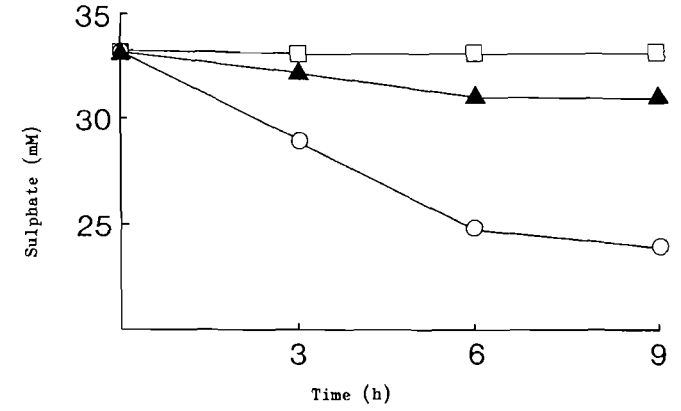

Figure 1, a

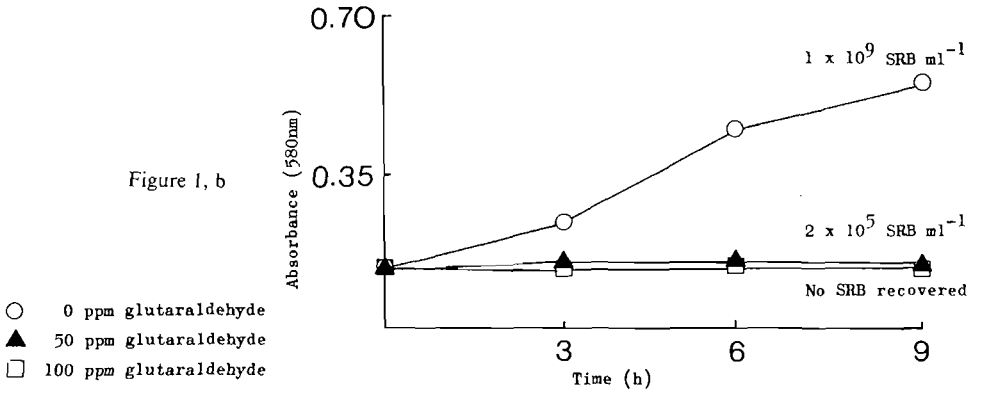

Figure 1, b

Figure 1 : Action of glutaraldehyde on sulphate reduction and growth of *Desulfovibrio desulfuricans NCIB 8400.*

A knowledge of the respiratory activity of SRB in the presence of an inhibitor is essential as most of their destructive properties are caused by their respiration of sulphate to sulphide. Figure 1 shows clearly that whilst 50 ppm glutaraldehyde inhibits SRB growth (Fig. 1b), sulphate reduction still occurs at a reduced rate (Fig. 1a). Complete inhibition of sulphide production is only achieved at a glutaraldehyde concentration of 100 ppm.

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