# The Malthus system for biocide efficacy testing against *Desulfovibriodesulfuricans*

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## Abstract

Microbially influenced corrosion (MIC) makes an important contribution to corrosion in various industries. Considerable success has been achieved by the use of biocides. Little information for controlling MIC is, however, available on the effectivity of biocides against sulphate-reducing bacteria (SRB) due to the difficulties of culturing these organisms using conventional techniques. Conductance changes monitored using the Malthus system were evaluated as an alternative method of estimating numbers *of Desulfovibrio desulfuricans* for laboratory biocide evaluations. The correlation of $\log_{10}$ counts *of Desulfovibrio* cells in iron sulphite (IS)-medium using conventional techniques with detection times using the Malthus systems was highly significant (r = 0,974), indicating that the Malthus system can be used as an alternative method to conventional media for the enumeration of SRB. Growth studies of *Desulfovibrio* using the Malthus system were useful in the evaluation of biocides. A 56 % and a 100 % kill was obtained when using 60 and 200 mg/t quaternary ammonium compounds (QAC), respectively.

## Introduction

The importance of dissimilatory SRB in MIC has been widely recognised for many years. Whilst their role in the sulphur cycle is fundamental in maintaining our environment, the adverse economic consequences of their activities can be devastating in industrial processes. These bacteria can cause health hazards and corrosion of equipment and pipelines (Boivin and Costerton, 1991; Crombie et al., 1980; Ford and Mitchell, 1990; Hamilton, 1985). The detection and monitoring of SRB in industrial water systems as well as their control by making use of biocides are therefore important to the industry.

The use of biocides to control biofouling in industrial water systems is an accepted practice (Cloete et al., 1992). However, incorrect use of biocides gives rise to biofouling and resistance developement in bacteria (Brözel and Cloete, 1991). It is therefore essential to select the correct biocide or combinations and their respective concentrations for the organisms to be killed. There are a variety of techniques for determining the effectivity of biocides (Cloete et al., 1990; Hilletal., 1989). Little published information is, however, available on the effectivity of biocides against SRB (Sharma et al., 1987).

There are many culture media formulations available that can be used for enumerating SRB (Ferodak et al., 1987; Pankhurst, 1971; Pfennig et al., 1981; Postgate, 1984). The preparation of anaerobic media is difficult and laborious (Gaylarde and Cook, 1987). It has been recommended that media should be incubated for up to 28 d (Herbert and Gilbert, 1984). Alternative methods such as antibodies (Bobowski and Nedwell, 1987; Gaylarde and Cook, 1987; Odom et al., 1991), have a low sensitivity. The high cost involved in using nucleic acid probes (Amann et al., 1990; Amann et al., 1992) and antibodies limit their use in the industry as well as in routine evaluations of biocides in the laboratory. Because of the difficulties associated with the enumeration of SRB, biocide evaluations against SRB have been neglected in the past.

Electrical methods (conductance, impedance and capacitance) are established methods for monitoring microbial growth and estimating bacterial numbers (Richards et al., 1978). One such system (Malthus) is based on the automated monitoring of electrical conductance in growing bacterial cultures. Conductance is measured by the introduction of platinum electrodes in the medium and the application of a low frequency voltage. When conductance values increase beyond a threshold value, these are recorded by the system and displayed graphically. The change detected in conductance is due to the metabolism of the constituents of the culture medium by the organisms. The time lapse between inoculation and a noticeable change in conductance is termed the detection time. Detection time is inversely proportional to the logarithm of the number of viable organisms inoculated into the medium assayed so that the instrument can be used for determining bacterial numbers (Gibson, 1985). Gibson (1987) used conductance measurements (Malthus Instruments, LTD Stoke and Trent, UK) to detect the growth of *Clostridium botulinum* in a selective medium. This indicated that the Malthus system had successfully been used for enumerating bacteria using selective media.

Therefore the technique of monitoring conductance changes using the Malthus system was evaluated as an alternative method of estimating numbers of *Desulfovibrio desulfuricans* for laboratory biocide evaluations. Not all culture media may be appropriate for conductive measurements (Gibson, 1987). Iron sulphite-medium (Mara and Williams, 1970) was chosen for these experiments, since this medium yielded the highest numbers when counting pure cultures of *D. desulfuricans* in studies comparing this medium with other generally used culture media for SRB (De Bruyn and Cloete, 1993).

Quaternary ammonium compounds are generally used in water water cooling systems for the control of algae. It would therefore be of interest to know what the effect of QAC would be against SRB in these systems. This was used as a model compound for evaluating this technique.

## Materials and methods

### Test organism

*Desulfovibrio desulfuricans* subsp. *desulfuricans* (DSM 1924) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM).

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