

ELECTRICAL ENHANCEMENT OF BIOCIDES ACTION FOR IMPROVED BIOFOULING AND BIOCORROSION CONTROL

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ABSTRACT

Attached microbial communities or biofilms cause a significant proportion of the fouling and corrosion problems faced by industry. Unfortunately, bacteria within the exopolysaccharide matrix of a biofilm are very well protected from antimicrobial agents such as biocides. Biofilm bacteria are generally resistant to 50 to 500 times the biocide concentration sufficient to kill planktonic or free-floating cells of the same species. We have recently discovered that the application of a low strength electric field/current density can enhance the efficacy of three common industrial biocides to such an extent that biofilm bacteria are killed at biocide concentrations lower than the planktonic minimum inhibitory concentration (MIC). This enhancement of biocide efficacy in the presence of an electric field/current density is known as the "bioelectric effect". By exploiting this effect it may be possible to significantly reduce biocide levels and their impact on the environment, without sacrificing their performance.

INTRODUCTION

Microorganisms are commonly found attached to surfaces in aqueous industrial systems. When these attached or sessile microorganisms produce a blanketing layer of exopolysaccharide, they are termed a "biofilm" (3, 9). These biofilms are often responsible for the fouling (7, 11, 13) and corrosion (1, 2, 4, 5, 6, 18) problems faced by industry. It is also widely acknowledged that biofilms within water distribution systems may act as reservoirs for pathogenic bacteria (eg. *Legionella pneumophila*) that pose a threat to the general population (16, 17, 19).

In industrial systems, microorganisms may be found as free-floating or planktonic cells, which are quite susceptible to the action of killing agents. However, when these cells attach to surfaces and form biofilms, their resistance to biocides increases quite markedly (12, 15, 19). The practical consequence to this resistance is that it is absolutely essential to sample not only a bulk water sample to ensure planktonic cell kill, but also to sample the pipeline wall to ensure that the attached population has been eradicated. Unless this protocol is followed, the pipeline will be continuously re-inoculated by cells from the more resistant biofilm population.

Even with the above protocol in place, satisfactory control of biofilm populations may not be achievable if the costs of the necessary biocide applications are not economically feasible. Recent studies in the medical section of our laboratory have found that

antibiotics work more effectively when applied against biofilms in a low strength electric field/current density (10). We have discovered that the efficacy of the three common industrial biocides tested to date can similarly be dramatically enhanced against biofilm bacteria, when applied in the presence of a low strength electric field/current density.

In this paper, we will present representative findings for the biocide glutaraldehyde against an environmental *Pseudomonas aeruginosa* biofilm.

METHODS

Biofilms were grown by flowing an environmental isolate of *Pseudomonas aeruginosa* in M-56 nutrient medium (8) through parallel perspex chambers which contained sampling studs of known diameter (0.5 cm²). These perspex chambers are called modified Robbins devices (MRDs)(14). One MRD was a non-electrified control and the other was electrified by connecting it, where stated, to a 3 V DC power source. Polarity was altered every 64 s, so that the electrodes alternated as anode and cathode.

The first experiment was performed to determine the effect of the electric field/current density on an established biofilm population. After a 24 h colonization period in the absence of an electric field/current density, the inoculum flask was replaced by one containing the nutrient medium M-56, and one MRD was electrified.

The second experiment was performed to determine whether biocide effectiveness was enhanced in the presence of an electric field/current density. The experimental protocol was identical, with the exception that the inoculum flask was replaced after a 24 h colonization period with one containing 5 ppm glutaraldehyde in M-56 nutrient medium.

Studs were sampled in duplicate from each device over the 24 h period following electrification. After sonication to remove the attached cells from the studs, the suspension was plated onto ½ Brain Heart Infusion plates. Results are reported as CFU/cm² after 48 h at 37°C.

RESULTS

The electric field/current density was of such a low strength that it did not have a significant effect on the established biofilm bacterial population when exposed in the absence of biocide. Viable cell counts were very similar between the control and electrified MRDs (Fig. 1).

Glutaraldehyde effectiveness was dramatically enhanced in the presence of the electric field/current density. The established biofilm bacterial population was killed within 24 hours (Fig. 2). This result was in sharp contrast to the non-electrified control, in which the level of glutaraldehyde employed had very little effect on the biofilm population over 24 hours (Fig. 2).

DISCUSSION

It is quite clear from these results that the biocidal action of glutaraldehyde can be significantly enhanced when applied in a low strength electric field/current density. It is also quite significant that this kill was achieved when using a glutaraldehyde concentration of only 5 ppm. This concentration is insufficient to kill the more sensitive planktonic bacterial cells.

This work and similar results from the two other common industrial biocides tested to date indicate that it may be possible to use this "bioelectric effect" to reduce the levels of biocide currently used, without sacrificing their effectiveness. This technology may be particularly attractive to industry if there are environmental impact concerns over the biocide concentrations employed at present. Further experiments are in progress to elucidate the mechanism of the effect.

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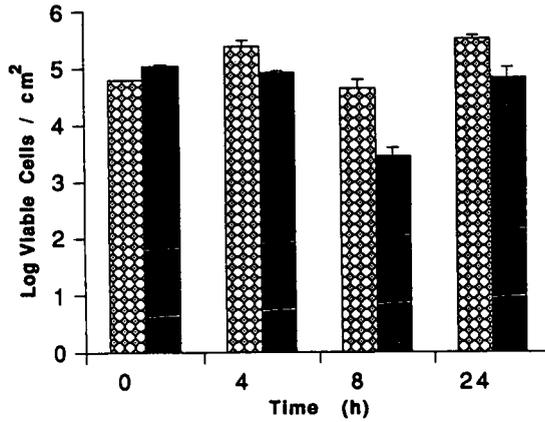


Figure 1 The effect of a low strength electric field/current density on an established *Pseudomonas aeruginosa* biofilm population. The control and electrified modified Robbins devices are represented by patterned and solid bars, respectively. (Mean \pm 1 SE, n=2).

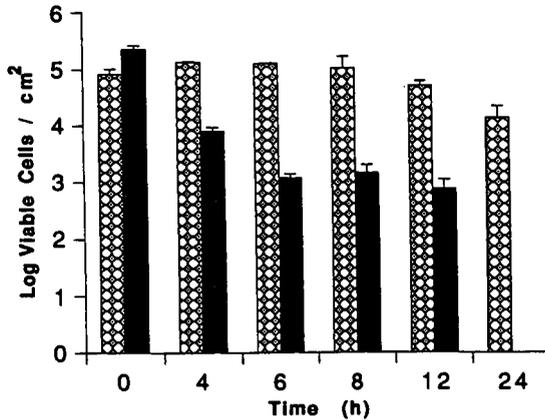


Figure 2 The effect of 5 ppm glutaraldehyde against an established *Pseudomonas aeruginosa* biofilm population in the absence (pattern) and presence (solid) of a low strength electric field/current density. (Mean \pm 1 SE, n=2).