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Electrical Enhancement of Biocide Efficacy against *Pseudomonas aeruginosa* Biofilms

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When applied within a low-strength electric field (±12 V/cm) with a low current density (±2.1 mA/cm^2), several industrial biocides exhibited enhanced killing action against *Pseudomonas aeruginosa* biofilms grown on stainless steel studs. Biocide concentrations lower than those necessary to kill planktonic cells of *P. aeruginosa* (1, 5, and 10 ppm of the active ingredients of kathon, glutaraldehyde, and quaternary ammonium compound, respectively) were bactericidal within 24 h when applied within our electrified device.

Bacterial biofilms have often been implicated in the corrosion (2–4, 14), souring or H_2S production (12, 13), and fouling (5, 9) problems experienced by industry. It has proven difficult to eradicate biofilms from pipelines because of their great resistance to bactericidal agents. The concentrations of biocides required to kill bacteria in the sessile phase are often much higher than those required for bacteria in the planktonic or free-floating phase (10, 15). Numerous hypotheses about the nature of this enhanced resistance in the sessile mode of growth have been suggested. Early hypotheses tended to center around the role of the abundant exopolysaccharide matrix of the biofilm. One hypothesis is that the exopolysaccharide matrix is a charged matrix and that this matrix is responsible for binding antimicrobial agents before they reach the target cells (7). We thought that it might be possible to disrupt the charges on the matrix sufficiently to allow penetration of bactericidal agents to the target cells by electrifying the system. The purpose of this study was to determine whether a variety of industrial biocides would exhibit enhanced action against *Pseudomonas aeruginosa* biofilms when applied within a low-strength electric field with a low current density (EF-CD).

(A preliminary description of this work was presented previously [1a].)

The biocides listed in Table 1 were first tested for their ability to kill planktonic cells of an environmental isolate of *P. aeruginosa*. This isolate was obtained from the Bow River (Calgary, Alberta, Canada) and was identified by M. Jacques (Université de Montréal). Sterile M-56 medium (6) was amended with an inoculum (2%, vol/vol) of *P. aeruginosa*. After 24 h, various concentrations of biocide (final concentration, 0 to 100 ppm of active ingredient), were added to the flasks. Samples were collected after 24 h at room temperature and plated onto half-strength brain heart infusion medium (1/2BHI). Results are reported as CFU per milliliter after 48 h at 37°C (Table 2). Biocide concentrations less than those necessary to kill planktonic cells (1, 5, and 10 ppm of the active ingredients of kathon, glutaraldehyde, and quaternary ammonium compound, respectively) were chosen for use in the biofilm experiments.

Electrified MRD. The modified Robbins device (MRD) (11) was further altered to determine whether biocide efficacy against biofilms could be enhanced in the presence of an EF-CD. A platinum wire electrode was incorporated into the device, and the stainless steel sample studs were converted into another electrode (Fig. 1). Each MRD held 12 sample studs (surface area, 0.5 cm^2) positioned flush with the inner surface to allow easy sampling of known areas of biofilm. The MRDs were run in parallel in each experiment (Fig. 2). One MRD was a nonelectrified control, and the other was electrified by connecting it, at the times stated, to a variable-voltage-and-current power source (maximums of 10 V and 50 mA, respectively). A constant potential of 3 V was applied which produced a field strength of ±12 V/cm and a current density of ±2.1 mA/cm^2. The polarity was altered every 64 s, so that the electrodes alternated as anode and cathode. A Cole-Parmer peristaltic pump controlled flow through the MRDs at 80 ml/h. The colonization culture was prepared by doing two transfers of the *P. aeruginosa* isolate (2%, vol/vol) into M-56 for 24 h at room temperature with stirring. Then 80 ml of this culture was added to 3.92 liters of M-56 medium immediately before connection to the MRDs. Two studs were collected at random from each MRD at each sampling time. The studs were individually sonicated (Branson 1200 low-output sonicator) for 2 min in 5 ml of phosphate-buffered

TABLE 1. List of biocides tested and their active ingredients

Code	Common name	Active ingredient
XC-215	Kathon	Isothiazalone (1.5%)
XC-507	Quaternary	Dimethyl ammonium chloride (50%)
XC-102	Glutaraldehyde	Glutaraldehyde (25%)

TABLE 2. Effect of various levels of biocide on a planktonic *P. aeruginosa* population

Biocide concn (ppm)	No. of CFU/ml with:		
	Kathon	Quaternary	Glutaraldehyde
0	3.0×10^8	3.0×10^8	3.0×10^8
1	4.0×10^2	ND[a]	ND
5	0	4.0×10^2	2.8×10^3
10	0	1.8×10^3	0
25	0	0	0

[a] ND, not determined.

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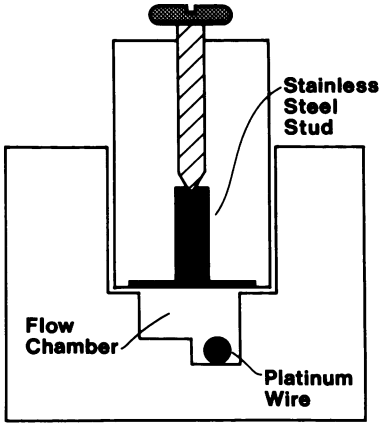

FIG. 1. Cross-sectional diagram of an electrified MRD showing the configuration of the perspex structure and the flow chamber and the relative position of the stainless steel stud and the platinum wire. Direct current was applied, with reversal of polarity each 64 s, to the stainless steel stud (via its retaining screw) and the platinum wire, which serve as the two electrodes needed to generate the EF-CD.

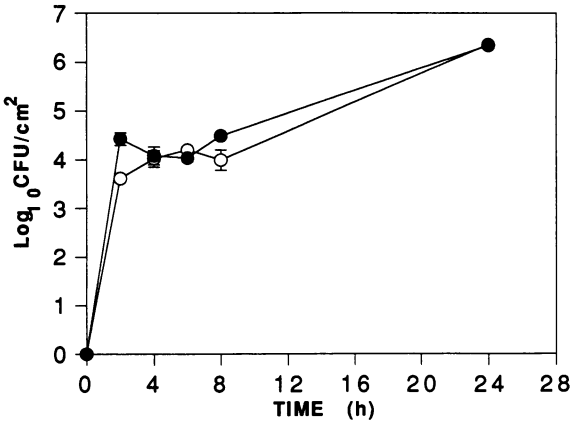

FIG. 3. *P. aeruginosa* colonization of stainless steel studs in the presence (●) and absence (○) of an externally applied EF-CD ($\bar{x} \pm$ 1 standard error, $n = 2$).

saline, vortex mixed, and plated onto 1/2BHI plates. Results are reported as numbers of CFU per square centimeter after 48 h at 37°C. Statistical analysis was performed with SuperANOVA software (1). All of the experiments had a significant ($P < 0.05$) interaction between the factors of electricity and time; therefore, contrast analysis was performed to determine significant differences (8). The experiment type I error rate was controlled to $\alpha = 0.05$ for all contrasts for a given effect.

Effect of the EF-CD on colonization. The *P. aeruginosa* reservoir was connected to an electrified MRD and a control MRD, and the studs were sampled over 24 h. There were no significant differences except at 2 h, when the number of CFU per square centimeter was significantly greater in the electrified MRD than in the control MRD (Fig. 3). Colonization was therefore not inhibited in the electrified MRD.

Effect of the EF-CD on an established (24-h) biofilm. After the MRDs were allowed to colonize for 24 h in the absence of an EF-CD, the *P. aeruginosa* reservoir was replaced with M-56 medium and one MRD was electrified. At 8 and 24 h, the number of CFU per square centimeter was significantly lower in the electrified MRD than in the control by approximately 1 log unit (Fig. 4). The EF-CD therefore had a detrimental effect on an established biofilm.

Effect of the EF-CD on biocide efficacy. In the first set of experiments, the *P. aeruginosa* reservoir was connected to an electrified MRD and a control MRD. As in the earlier trial, the EF-CD did not have a detrimental effect on colonization. In fact, the number of CFU per square centimeter was significantly greater in the electrified MRDs at 24 h (Fig. 5A).

At 24 h, the *P. aeruginosa* reservoir was replaced with M-56 medium containing glutaraldehyde or kathon (Fig. 5A). Glutaraldehyde did not have a significant effect on the number of CFU per square centimeter in the control MRD. In the electrified MRD, however, there was a significant decrease between 24 and 48 h. Counts were significantly lower in the electrified MRD than in the control MRD from 36 h onward. The action of glutaraldehyde was therefore enhanced when applied in an EF-CD. Similar results were found for the kathon trial. The number of CFU per square centimeter decreased significantly between 24 and 37 h in the control MRD, but this decrease was not as rapid as that in the electrified MRD. The number of CFU per square centimeter was significantly lower in the electrified MRD than in the control MRD from 30 h onward.

A second set of experiments was performed to determine whether this enhanced biocide effect was dependent on whether the biofilms had been established in the presence of an EF-CD. The above experiment was repeated on devices that had been colonized for 24 h in the absence of an EF-CD.

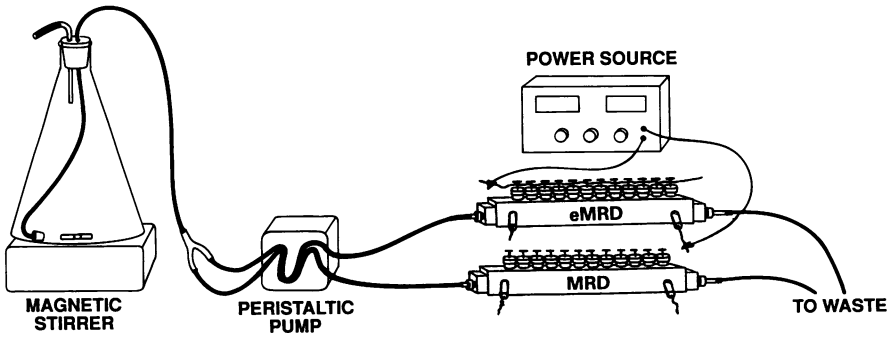

FIG. 2. Diagrammatic representation of the MRD, the electrified MRD (eMRD), and the apparatus used to provide a continuous flow of bacterial inoculum, nutrient medium, or biocide-amended nutrient medium.

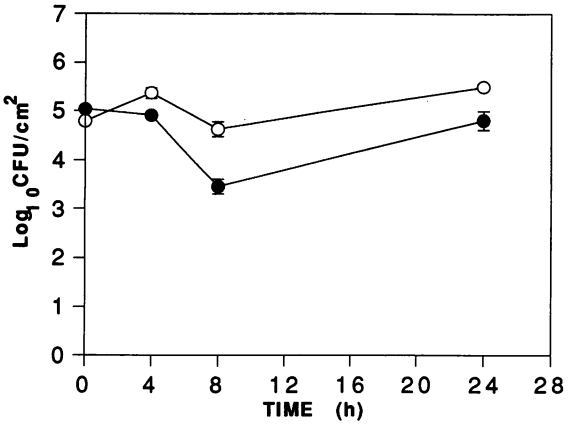

FIG. 4. Effect of an EF-CD on an established (24-h) *P. aeruginosa* biofilm. Symbols are as in Fig. 3.

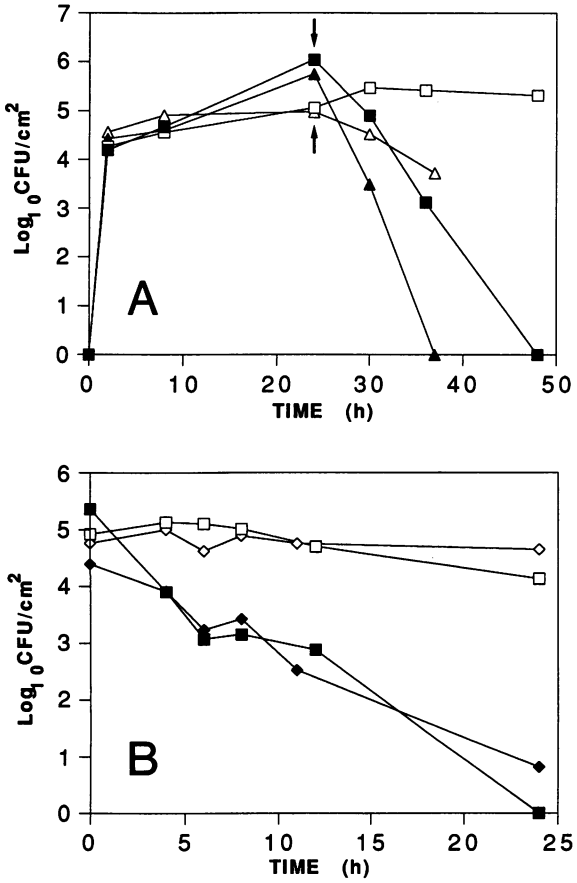

FIG. 5. (A) Effect of an EF-CD followed by biocide application (arrows) on *P. aeruginosa* colonization ($\bar{x}$, n = 2). At 24 h, glutaraldehyde (5 ppm) (□, ■) or kathon (1 ppm) (△, ▲) was applied to both electrified and control devices. (B) Effect of biocides on an established (24-h) *P. aeruginosa* biofilm in the presence and absence of an EF-CD. Glutaraldehyde (5 ppm) (□, ■) or quaternary ammonium compound (10 ppm) (◇, ◆) was supplied to both electrified and control devices for 24 h ($\bar{x}$, n = 2). The electrified devices are represented by solid symbols (■, ▲, ◆).

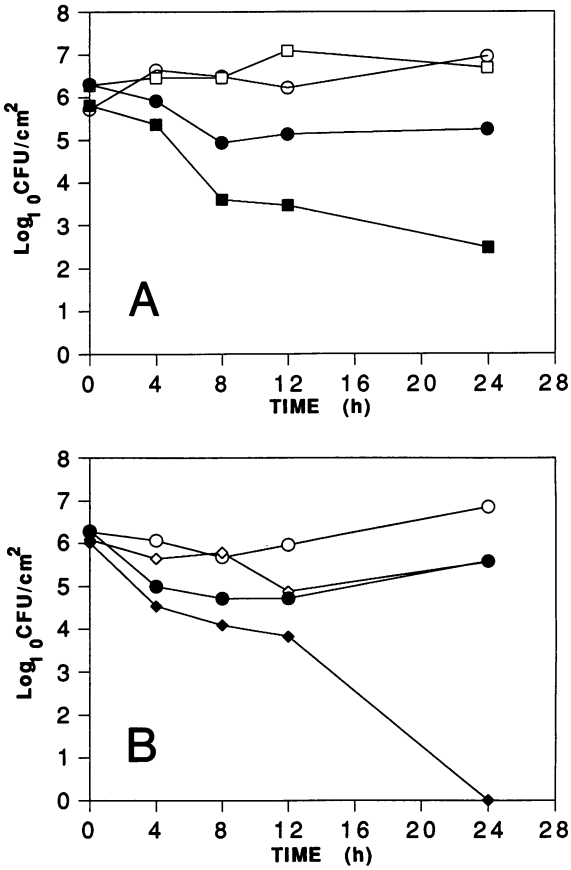

FIG. 6. Effect of biocide and/or electrification (3-V EF-CD) treatments on established (24-h) *P. aeruginosa* biofilms. Samples were collected from electrified (■, ●, ◆) and nonelectrified (□, ○, ◇) devices in the absence of biocides (●, ○) or in the presence of glutaraldehyde (5 ppm) (A) (■, □) or quaternary ammonium compound (10 ppm) (B) (◆, ◇) ($\bar{x}$, n = 2).

In the glutaraldehyde trial, the number of CFU per square centimeter was significantly lower in the electrified MRD from 4 h onward (Fig. 5B). In the quaternary ammonium compound trial, the number of CFU per square centimeter in the electrified MRD was significantly lower from 8 h onward. The enhancement of biocide efficacy was therefore not dependent on the conditions (presence or absence of an EF-CD) under which the biofilm was established.

The final set of experiments was performed to demonstrate the relative contributions of the EF-CD and biocide (glutaraldehyde or quaternary ammonium compound) to the overall effect. Established (24-h) biofilms were monitored under simultaneous (i) biocide treatment, (ii) electrification, (iii) combined biocide and electrification, and (iv) no biocide or electrification (control).

In the glutaraldehyde trial, the combined biocide and electrification resulted in numbers of CFU per square centimeter that were significantly lower than those with the biocide or electrification treatment from 8 h onward (Fig. 6A). At 24 h, the numbers of CFU per square centimeter in the control and biocide treatments were not significantly different because of the low level of biocide used. However, this same biocide concentration used in the electrified device resulted in numbers of CFU per square centimeter that were

significantly lower than those due to electrification or to biocide treatment alone.

In the quaternary ammonium compound trial, the combined biocide and electrification treatment resulted in numbers of CFU per square centimeter that were significantly lower than those with the biocide or electrification treatment from 4 and 12 h onward, respectively (Fig. 6B). The synergistic effect of biocide and the EF-CD was clearly demonstrated at 24 h. When the biocide and electrification treatments were applied separately, the number of CFU per square centimeter was approximately 1 log unit lower than that in the control. When these treatments were combined, however, a complete kill was obtained.

In summary, this paper presents our initial findings that three common industrial biocides exhibit enhanced action when applied against *P. aeruginosa* biofilms within a low-strength EF-CD. The nature of the mechanism(s) has yet to be confirmed, as a number of mechanisms such as electroporation, electrophoresis, and iontophoresis could be responsible. If this bioelectric effect holds true for a wide variety of biofilms, biocides, and surfaces, this technology will have great promise in circumstances where high biocide concentrations are impractical, uneconomic, or environmentally hazardous and prohibited by law. Further studies are in progress.

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