

A Superior Biocide for Disinfecting Reverse Osmosis Systems

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*In an effort to determine the effectiveness of Minncare® as a sterilant compared to biocides traditionally recommended for use on reverse osmosis (RO) membranes and their associated water distribution systems, Minncare® Cold Sterilant, formaldehyde, bleach and hydrogen peroxide were tested for efficacy by an independent testing laboratory. The concentrations chosen for the test were those normally recommended for cleaning and sanitizing RO membranes and water systems. Survival curves were generated for each biocide against *Bacillus subtilis* var. *niger* (ATCC 9372) spores. These provided data for calculating individual D-values. A D-value is the time required to reduce the microbial population by 1 log. A D-value is generally accepted as the time required for disinfection. Results show that Minncare® Cold Sterilant exhibited a 6 D-value of 36 minutes, compared to 11 hours for formaldehyde, 7 hours for bleach, and 1 to 25 hours for hydrogen peroxide.*

Normal maintenance of RO membranes and water distribution systems requires routine cleaning and disinfection to prevent bacterial growth. Microbial contaminants commonly found in feedwater will decrease the operating efficiency of RO membranes, lower membrane flux and thereby increase the cost of producing high purity water. Fouling microorganisms may actually destroy some RO membranes by promoting decomposition of the membrane polymer. Bacterial growth acceleration, particularly during periods of shutdown, requires a microbiocidal agent to control growth and protect the water system.

Organic and inorganic fouling of the RO membrane can be removed with cleaning agents. Sanitization, which must achieve a 3 log reduction of bacteria,¹ is usually accomplished post-cleaning to eliminate fouling microorganisms. Since naturally occurring microbial contaminants on the membrane can easily reach levels of 10⁴ colony forming units per square centimeter (CFU/cm²),² sanitization may not completely destroy all the microbial bioburden present. Disinfection, requiring a 6 log reduction of bacteria³, may be more appropriate. The biocides historically used for maintenance of RO membranes and their water distribution systems include formaldehyde, bleach, and hydrogen peroxide.⁴ Hydrogen peroxide

requires either extended soak times at low concentrations or high concentrations which may be destructive to the RO membrane. The high concentrations, however, are necessary for adequate disinfection of the water distribution system. Bleach, while suitable for disinfection of cellulose acetate membranes, is contraindicated for thin-film composite membranes. Formaldehyde, while compatible with both thin-film composite membranes and water distribution systems, present toxicity and environmental hazards. Additionally, chemicals of the formaldehyde family, although effective for destroying microbial contaminants, do not remove the extracellular material associated with biofilms. This can result in a more rapid re-fouling of the water system.⁵

Minncare® Cold Sterilant, a proprietary, stabilized mixture of hydrogen peroxide and peracetic acid, is an oxidizer compatible with thin-film composite membranes. Oxidizers are known to not only kill bacteria but also effectively remove biofilms⁵. In order to evaluate the efficacy of Minncare® versus other biocides historically used for RO and water system maintenance, the following study was undertaken.

* Employed by Minntech Corporation, Minneapolis, MN at time of study.

** Employed by FilmTec Corporation, Minneapolis, MN at time of study.

Materials and Methods

Test Organism

A *B. subtilis* var *niger* (ATCC 9372) aqueous spore suspension (North American Science Associates, Inc.) was used as the test bioburden. While spore-forming bacteria are not the predominant microorganisms associated with RO systems, they are highly resistant to disinfectants and were therefore chosen as the challenge organism for this study. A sufficient concentration of spores, in 0.1 milliliter (ml) volumes, was added to the following biocides to achieve a final concentration of 2×10^6 spores/ml: Minncare® at 1% concentration, formaldehyde at 2% concentration, sodium hypochlorite (bleach) at 0.001% concentration, and hydrogen peroxide at 0.2%, 5%, and 10% concentrations. Biocide/ spore mixtures were equilibrated to 20°C.

Aliquots from each biocide/spore mixture were removed for analysis of surviving spores at 0, 15, 30, and 60 minutes, and 2, 4, 6, 12 and 24 hours.

Each sample was neutralized, as described below, and then diluted and plated (0.1 ml of each neutralized sample and dilutions as necessary) on Tryptic Soy Agar using the pour plate technique. Plates were incubated at 35°C for 7 days. Colonies derived from surviving spores were counted and reported as CFU/ml. It should be noted that because 0.1 ml samples were analyzed, the minimum number of survivors detectable per ml was 10 CFU.

Neutralization

The activity of each biocide was neutralized at the time each aliquot was sampled to ensure that survivors detected at each time interval accurately represented the killing power of the biocide for that exposure. The neutralizing agents used are set forth in Table 1.

Table I
Neutralizing Agents and Concentrations

Biocide	Neutralizing Agent	Final Concentration ¹
Minncare® disinfectant	D/E Neutralizing	100X dilution
Formaldehyde	Sodium bisulfate	1% Aqueous
Sodium hypochlorite	Sodium thiosulfate	Equimolar
Hydrogen peroxide	D/E Neutralizing broth ²	100X dilution

¹Final concentration of neutralizing agent when combined with biocide/spore sample.

²DIFCO, Cat #0819-17-2

Validating The Neutralization

A separate test was done to validate the effectiveness of the neutralizers chosen. Test biocides, at use-concentration and equilibrated to 20°C, were sampled at the time intervals selected for efficacy testing, and each sample was mixed with the appropriate volume and type of neutralizing agent to achieve the final concentrations given in Table 1. A 0.1 ml sample of each biocide/neutralizer mixture was plated using the

pour plate technique and Tryptic Soy Agar. Ten *B. subtilis* spores were then added to each plate. Controls were set up without biocide or neutralizer, to ensure that neutralizers were not toxic to the spores. Plates were incubated at 35°C for 7 days. At the end of 7 days, all plates showed recovery of all *B. subtilis* spores added, demonstrating in each case that neutralization was achieved.

Generating D-Values

A D-value was generated for each biocide tested. The log of the number of surviving spores at each time frame tested was plotted and a "best fit" line generated by log regression analysis. The slope of the regression line in each case was determined. The negative reciprocal of the slope of each line is the D-value in minutes. A 6 D-value is determined by multiplying the D-value by 6 to give the time required to achieve a 6 log reduction of the spore population. Since the 5% and 10% hydrogen peroxide efficacy data showed a 1 log reduction in 60 and 30 minutes respectively, but <10 CFU/ml thereafter, use of the data only up to the 60 and 30 minute time frames respectively would give a D-value far larger than that actually reflected by the data. A value of "0" for the <10 CFU/ml could not be used, since the log of 0 is undefined. Therefore, a value of 10 CFU/ml was

assigned at the 60 minute time frame for 5% hydrogen peroxide. This was not necessary for the other biocides tested because there was sufficient data covering at least 3 log reductions of the spore population to generate a reliable D-value.

Results

Sterilant Efficacy

The results of efficacy testing are shown in Table II. Minncare® Cold Sterilant, at a 1% concentration showed a 5 log reduction of bacterial spores in 30 minutes. By contrast, 2% formaldehyde required 12 hours and 0.001% sodium hypochlorite 6 hours to achieve a 5 log reduction. Hydrogen peroxide, at 10%, 5%, and 0.2% concentrations, required approximately twice the amount of time as Minncare® Cold Sterilant and as much as 24 hours to exhibit the same 5 log reduction.

**Table II
Biocide Efficacy Results**

Exposure Time	Minncare® (1%)	Formaldehyde (2%)	Sodium hypochlorite (0.001%)	Hydrogen peroxide (0.2%)	Hydrogen peroxide (5%)	Hydrogen peroxide (10%)
0 minutes	2.3 x 10 ⁶	2.3 x 10 ⁶	2.1 x 10 ⁶	2.2 x 10 ⁶	2.1 x 10 ⁶	2.0 x 10 ⁶
15 minutes	1.1 x 10 ⁶	2.3 x 10 ⁶	2.2 x 10 ⁶	2.3 x 10 ⁶	2.0 x 10 ⁶	2.0 x 10 ⁶
30 minutes	3.0 x 10 ¹	2.1 x 10 ⁶	1.1 x 10 ⁶	2.3 x 10 ⁶	2.0 x 10 ⁶	2.0 x 10 ⁵
60 minutes	<10	2.0 x 10 ⁶	4.0 x 10 ⁴	2.0 x 10 ⁶	1.0 x 10 ⁵	<10
2 hours	<10	1.5 x 10 ⁵	1.0 x 10 ⁴	2.0 x 10 ⁶	<10	<10
4 hours	<10	1.2 x 10 ⁵	1.0 x 10 ³	1.5 x 10 ⁶	<10	<10
6 hours	<10	1.0 x 10 ³	<10	1.0 x 10 ⁶	<10	<10
12 hours	<10	<10	<10	1.0 x 10 ³	<10	<10
24 hours	<10	<10	<10	<10	<10	<10
D-values	6 minutes	113 minutes	69 minutes	250 minutes	22 minutes	11 minutes
6D	36 minutes	678 minutes	414 minutes	1500 minutes	132 minutes	66 minutes

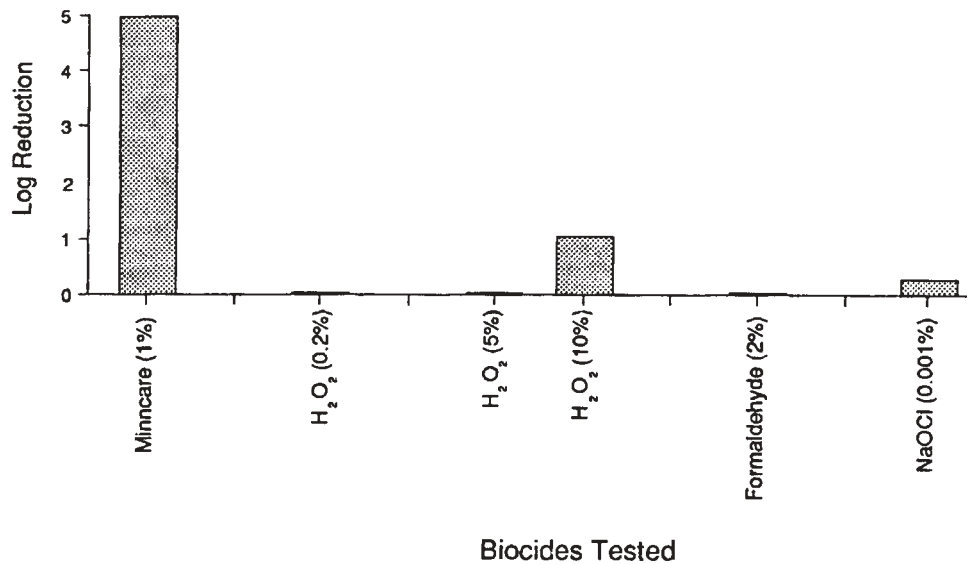


Figure 1: Log reduction of (ATCC 09372) Spores after 30 minutes Exposure to Various Biocides.

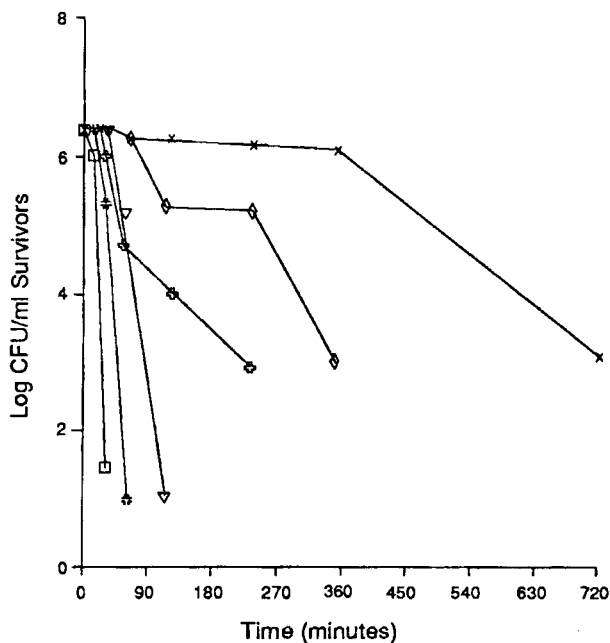


Figure 2: Survival Curves for Biocides Tested for Efficacy by Suspension Test against *B. subtilis* var *niger* (ATCC 9372) Spores. □ = Minncare (1%); X = Hydrogen peroxide (0.2%); ▼ = Hydrogen peroxide (5%); ⊕ = Hydrogen peroxide (10%); = Formaldehyde (2%); + = Sodium hypochlorite (0.001 %).

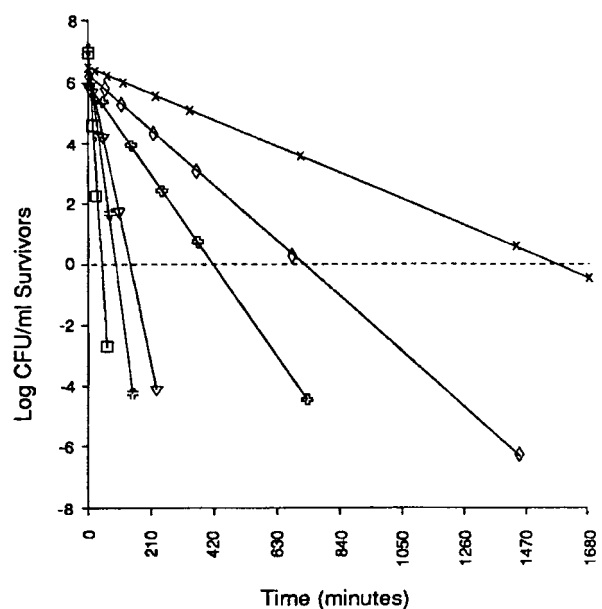


Figure 3: Log Regression Lines from Analysis of Survival Curves for Biocides Tested for Efficacy by Suspension Test against *B. subtilis* var *niger* (ATCC 9372) spores. □ = Minncare (1%); X = Hydrogen peroxide (0.2%); ▼ = Hydrogen peroxide (5%); ⊕ = Hydrogen peroxide (10%); = Formaldehyde (2%); + = Sodium hypochlorite (0.001 %).

Survival Curves, Regression, Analysis, and D-Values

Survival Curves for each biocide, based on the log of the number of CFU/ml of spores surviving at various exposure times, is presented in Figure I. The regression lines generated from each survival curve are also plotted on the same axes for each biocide. The D-values, resulting from the regression analysis as described in the Material and Methods section, reflect the diversity of effectiveness seen among the biocides tested. The D- values are listed at the bottom of Table II.

Minnicare® Cold Sterilant had the smallest D-value of 6 minutes, while the other test biocides gave D-values ranging from 11 minutes for 10% hydrogen peroxide to 69 minutes for sodium hypochlorite, and 113 minutes for formaldehyde.

The 6 D-value is an indicator of the time necessary for a 6 log reduction in the bacterial population, giving a 1 in 6 log probability of having an organism survive the disinfection cycle.

Minnicare® Cold Sterilant, with a 6 D-value of 36 minutes, achieved disinfection in the shortest period of time. The other biocides required between 66 minutes and 25 hours (1500 minutes) to achieve the same 6 log reduction.

Conclusions

A 1% concentration of Minnicare® Cold Sterilant was the most effective biocide tested, compared to other commonly used biocides. A 6 log reduction of B subtilis spores was achieved in less than one hour only with Minnicare® Cold Sterilant. Formaldehyde, hydrogen peroxide, and bleach all required longer exposure times. Minnicare® Cold Sterilant can be used to disinfect both the water distribution system and RO membranes at a 1% concentration with excellent results. Both thin-film composite and cellulose acetate membranes are compatible with Minnicare® Cold Sterilant. Using Minnicare® Cold Sterilant eliminates problems commonly associated with hydrogen peroxide and formaldehyde.

References

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