Bacteriological Evaluation of an Ultra-Pure Water-Distilling System

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A prototype distillation and storage system with recycle for producing ultrapure water was monitored for bacteriological contamination during a period of 24 months. Naturally occurring Pseudomonas aeruginosa and P. cepacia were found to grow rapidly to levels of about 10^4/ml in water taken from the storage reservoir and also in commercially prepared distilled water. The system was found to eliminate bacterial contaminants introduced into the still with the feed water, but the reservoir, once contaminated, remained contaminated during prolonged recycle. After a single treatment with free chlorine, the entire system remained uncontaminated until accidental or purposeful shutdown.

Favero et al. (6) reported the detection of relatively high levels of gram-negative bacteria, including Pseudomonas aeruginosa and fecal coliform bacteria, in the distilled-water reservoirs of mist therapy units used in a hospital pediatric ward. Following this report, other workers responded with observations of and possible solutions to the potential hazards manifested by contaminated and carelessly handled distilled waters in hospitals (1, 3, 4, 7-10). Hickman et al. (9) reviewed the evolution of conventional distillation systems and described their efforts to produce ultra-pure water and to maintain its physical and biological integrity. Recognizing that absolute purity of water is unattainable and stored samples quickly deteriorate, their approach was to regroup conventional equipment into a prototype still using a combined still-storage reservoir system in which product water was continuously recycled back to the still for redistillation. With the prototype distillation system, it was possible to produce pyrogen-free water with up to 91.5% of the electrical resistance of pure water while at the same time maintaining an uncontaminated state in the system for long periods of normal in-use operation. This report describes the continuing analysis and upgrading of the physical and biological parameters of the prototype still and its product water.

Past studies (6) indicated that when fresh, high-quality distilled water was allowed to remain in contact with air for a few days, the aged water would support better growth of naturally occurring P. aeruginosa than would the fresh water. Presumably, this was due to absorption of volatile or gaseous nutrient materials during exposure so the concentrations of these nutrients were reflected in increased growth rates and maximum cell populations obtained. It was of interest, therefore, to determine the comparative "qualities" of fresh and aged water (RITW) from the prototype still in parallel with fresh and aged commercially distilled water (CDW) used in hospitals.

RITW was transferred from the still reservoir to a chemically cleaned and sterilized 1-liter borosilicate flask. The flask was flame sealed after filling and, after 1 week, was opened by aseptic technique in a horizontal laminar-flow bench (5). Four 50-ml samples were poured into sterile, chemically clean 125-ml Erlenmeyer flasks sealed with aluminum foil, and two 10-ml portions were withdrawn for use as diluent. Similarly, a bottle of CDW was opened and samples were withdrawn as above. Cultures of naturally occurring P. aeruginosa (1) and of naturally occurring P. cepacia (2) maintained at 25 C in CDW were diluted in RITW and CDW and used to inoculate paired flasks of freshly opened RITW and CDW, respectively. The remaining RITW in the liter flask (about 800 ml) was inoculated with P. cepacia (<1 cell/ml), resealed, and incubated at 37 C to be used later to contaminate the reservoir and still. All cultures were incubated at 37 C, and viable counts were determined at selected intervals from pour plates, using standard methods agar (Difco). The remaining two RITW and CDW flasks were stored at 25 C for 1 month and then were inoculated and assayed as above to determine the effect of storage ("aging") on water quality.

Growth curves for P. cepacia and P. aerugi-
nosa are shown in Fig. 1. The culture of P. cepacia rapidly obtained a plateau population of $1.5 \times 10^6$ cells/ml in "fresh" RITW and CDW, whereas P. aeruginosa leveled off at somewhat lower populations of $4 \times 10^5$ to $7 \times 10^5$ cells/ml. Growth curves for P. cepacia were essentially the same in both RITW and CDW, with generation times (time required for the population to double) of 2 h. With P. aeruginosa, the generation time was somewhat greater in RITW (13.3 h) than in CDW (8.3 h), but maximum levels of cells per milliliter were equivalent. Cells of P. aeruginosa and P. cepacia in "aged" RITW and CDW obtained population levels of $2.1 \times 10^6$ cells/ml within 30 h and had generation times of 1 h in both waters. Using these test systems, it was impossible to compare "purity levels" of the waters with respect to the ability to support microbial growth; however, it is obvious that the potential for rapid growth of both organisms existed in both waters, and, if exposed to the atmosphere, this potential increased and allowed massive contamination levels to be reached within 24 h. Implications of this contamination have been discussed (3).

To determine whether the distillation system was self-cleaning, the still reservoir was purposely contaminated with a culture of naturally occurring P. cepacia. The reservoir had previously been decontaminated with chlorine vapors (procedure described below) and was shown to be uncontaminated by a series of bacteriological tests. One liter of water was withdrawn from the reservoir, and 200 to 250 ml of the resealed flask culture containing $\sim 5 \times 10^4$ P. cepacia/ml was introduced aseptically through the bottom. The bacteria were flushed into the reservoir with the previously withdrawn water until the normal reservoir level was established. Beginning at once and periodically thereafter, duplicate 10-ml samples from the 31 to 35 C bottom region of the reservoir were withdrawn aseptically into tubes containing 10 ml of double-strength Eugon broth (BBL) and incubated at 35 C for 2 weeks. The tubes were checked daily for turbidity; positive samples were streaked for isolation on Trypticase soy agar (BBL), and randomly selected colonies were identified biochemically (2) to confirm the presence of P. cepacia. No isolates other than P. cepacia were obtained. All samples taken during the initial 24 h showed turbidity, whereas only two out of eight samples taken from 48 to 120 h were positive for growth. This showed that the contamination level in the reservoir was lowered but not eliminated after 48

![Graph](image-url)  
**Fig. 1. Growth of Pseudomonas aeruginosa and P. cepacia in fresh and aged RITW (water from the prototype still) and CDW (commercially prepared sterile distilled water).**
h of reservoir-still refluxing. After 120 h of running time, a test was conducted to determine the location of the *P. cepacia* within the thermal density gradient of the reservoir (100 to 35 °C). Half of the water was drained from the reservoir, during which 10-ml samples were collected at ascending temperature levels to a maximum of 47 °C. No *P. cepacia* was detected in the region above 42 °C. Daily drainage of 1 liter of water, simulating in-use operation, and collection of 10-ml samples from the 37 °C zone into tubes of double-strength Eugon broth showed positive readings in eight of ten tubes taken over a 1-week period. Apparently, *P. cepacia* had been established in the lower region of the reservoir. The reservoir was then drained completely, and steam from the boilers was allowed to issue from the reservoir drain spout and the condensers for 10 to 15 min in an effort to decontaminate the reservoir. Bacteria on the wall of the vessel should have been exposed to a 100 °C environment, but, even after this treatment, *P. cepacia* was still present in samples taken from the 37 °C region after the reservoir had refilled with fresh distillate. The system appeared incapable of self-decontamination.

There was need for a simple and rapid means for decontamination of the reservoir that would not add massive chemical contamination as the earlier use of formaldehyde had done (9). A small chlorine gas generator was installed in the path of the steam from the demister to the purge boiler condenser (see Fig. 4 in ref. 9). Two milliliters of sodium hypochlorite solution (16%) and 1 ml of concentrated sulfuric acid were added to the generator; most of the Cl\(_2\) was released within 15 min and could not be detected after 2 h of steaming. The presence of chlorine in the distillate draining from the empty reservoir was tested for with orthotolidine. After the chlorine treatment, the residue in the chlorine generator was drained and the drain spout on the reservoir was closed. Bacteriological tests conducted after the reservoir had refilled and the conductivity readings had stabilized showed that the system was not contaminated.

To determine whether bacterial contamination could be carried by steam from the boiler into the reservoir, the boiler (1.2-liter capacity) was momentarily shut off and a 200-ml volume was replaced with 200 ml of the resealed flask culture of naturally occurring *P. cepacia* (~5 × 10\(^4\) cells/ml). The system was restarted, and samples of product water assayed at intervals for several weeks from the 37 °C level in the reservoir showed that there was no carryover of bacteria from the boiler to the reservoir.

Earlier tests by Hickman et al. (9) showed that the system remained uncontaminated for indefinite periods under normal operating conditions. This observation has been reconfirmed after installation of the in-line chlorine generator. The feasibility of producing a continuous supply of ultra-pure water (>14 μmos) free from bacterial contamination has been demonstrated. The applicability of such a system in, for instance, a hospital or specialized laboratory depends upon a larger engineered design of the system coupled with personnel cognizant of water quality measurement, periodic monitoring practices, and techniques of asepsis.

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