Journal of Industrial Microbiology, 8 (1991) 223–228
Elsevier

SIM 00367

Original Papers

Oligotrophic bacteria in ultra-pure water systems: media selection and process component evaluations

Robert A. Governal[1], Moyasar T. Yahya[2], Charles P. Gerba[2] and Farhang Shadman[1]

[1]*Department of Chemical Engineering, and* [2]*Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona, U.S.A.*

(Received 26 February 1991; revision received 30 May 1991; accepted 6 June 1991)

Key words: Deionized water; Ultra-pure water; Ozone; Ultra-violet sterilization; Oligotroph; Bacteria; R2A medium

SUMMARY

Presently, tryptic soy agar (TSA) medium is used in the semiconductor industry to determine the concentration of viable oligotrophic bacteria in ultra-pure water systems. Deionized water from an ultra-pure water pilot plant was evaluated for bacterial growth at specific locations, using a non-selective medium (R2A) designed to detect injured heterotrophic as well as oligotrophic bacteria. Results were compared to those obtained using Tryptic Soy Agar. Statistically greater numbers of bacteria were observed when R2A was used as the growth medium. Total viable bacterial numbers were compared both before and after each treatment step of the recirculating loop to determine their effectiveness in removing bacteria. The reduction in bacterial numbers for the reverse osmosis unit, the ion exchange bed, and the ultraviolet sterilizer were 97.4%, 31.3%, and 72.8%, respectively, using TSA medium, and 98.4%, 78.4%, and 35.8% using R2A medium. The number of viable bacteria increased by 60.7% based on TSA medium and 15.7% based on R2A medium after passage of the water through an in-line 0.2-μm pore size nylon filter, probably because of the growth of bacteria on the filter. Our results suggest that R2A medium may give a better representation of the microbial water quality in ultra-pure water systems and therefore a better idea of the effectiveness of the various treatment processes in the control of bacteria.

INTRODUCTION

In recent years, guidelines for ultra-pure water for industrial applications (e.g. semiconductor industry, pharmaceutical industry, etc.) have become increasingly more stringent. These guidelines include maximum acceptable levels of bacteria (Table 1). When viable or inactivated bacteria are present in water used in various manufacturing processes, they can interfere with the function of the final product. For example, in a semiconductor device, one bacterium can have enough phosphorus to cause a defect in a 64-kilobyte (64 KB) DRRAM (Dynamic Random Access Memory) device [12]. Viruses are capable of causing critical defects through irregular doping of the water substrate on a 4-megabyte (4MB) DRAM device [12].

The maximum acceptable levels for microorganisms require the water quality engineer to re-evaluate fundamental testing procedures involved in quantifying viable bacteria. Variables such as water temperature, sampling technique and media selection can be critical in obtaining accurate and reproducible results [7].

TABLE 1

Recommended water quality limits for circuit devices[a]

Integration scale	64KB	256KB	IMB	4MB
Resistivity (MΩ/cm)	15– 16	17– 18	17.5–18	18
Particles (counts/ml)	50–150	30– 50	0 –20	5–10
Critical particle size (μm)	0.2	0.2	0.1	0.1
Bacteria (cfu/100 ml)	50–100	5– 20	1 – 5	1
Total organic carbon (μg/l)	50–200	50–100	30– 50	20–30
Silicon dioxide (μg/l)	20– 30	10	5	5
Dissolved oxygen (μg/l)	100	100	50–100	50

[a]Adapted from Yabe et al. (1989).

Present procedures include sampling a known volume of water from the point of use and filtration through a 0.2-μm pore size microporous membrane (Nuclepore, Cambridge, MA). The filter is then placed on tryptic soy agar (TSA) and incubated for 72 h at 35 °C. Recommended incubation times and temperatures have ranged from 24 h to 1 week, and 25 to 35 °C [10]. These conditions do not take into account the nutrient-poor environment which exists in a high-quality water recirculating loop (or polishing loop) of a semiconductor fabrication device's water; the environment may place stress on bacteria which inhabit these environments [1]. In addition,

Correspondence: R.A. Governal, Department of Chemical Engineering, University of Arizona, Tucson, AZ 85721, U.S.A.

many of the organisms may be damaged by the various water treatment processes (e.g. disinfection). The use of TSA medium, and incubation under conditions other than the environment found in an ultra-pure water line (e.g. increased temperature) may cause an inaccurate count of viable bacterial numbers [1]. Bacteria found in ultra-pure water systems such as *Pseudomonas* spp. [7], display unique survival characteristics in an ultra-pure water environment compared to a waste water treatment system, and may require a non-selective medium such as R2A for optimal recovery of viable organisms [10].

The purpose of this study was to determine the need for using non-selective media with respect to numbers of injured and viable cells, and to consider the interactions between system components within the ultra-pure water polishing loop with respect to removing or recovering bacteria.

MATERIALS AND METHODS

Purification apparatus

The water purification system used in this study contained a primary feed, and a polishing loop (Fig. 1) [11]. The primary feed of the pilot plant used in this study consists of initial water conditioning by a water softener (replaces calcium ions in water with sodium ions), and a carbon bed filtration unit which removes chlorine from the water and acts a filter (5 μm nominal pore size). The water is then pumped through a reverse osmosis unit to reduce bacterial numbers in the permeate. The water then enters the polishing loop to remove organic matter and ions. Due to the stringent requirements of parts per billion (μg/l) organic contamination required for ultra-pure water, the polishing loop consists of 316 L electropolished stainless steel or PVDF (polyvinylidene fluoride) piping and housings [7]. The reverse osmosis (RO-15, Millipore, Bedford, MA) permeate enters the polishing loop via the PVDF storage tank and PVDF pump. The water is then passed through a mixed bed ion exchange tank (0.45-cubic foot tank, Millipore, Bedford, MA), a 0.04-μm pore-size nylon filter (Pall Posidyne N66, White Plains, NY), and an ultraviolet light sterilizer (SL-1-TOC, Aquafine, Valencia, CA) that produces both the germicidal 254-nm wavelength and a 185-nm wavelength capable of oxidizing carbon compounds. The water then circulates back into the 37.8-l storage tank. The water from this pilot plant is characterized as follows: resistivity is 18 MΩ-cm (25 °C), no greater than 1 particle per ml greater than 0.1 μm in size, less than 30 viable bacteria per liter, total oxidizable carbon less than 2.5 μg/l, and total silica less than 1 μg/l. During the second month of this study, ozone was added to the ultra-pure water to determine its effectiveness in inactivating bacteria [13].

Sample collection

Two 1-l samples were obtained from the pilot plant at specific locations over a 2-month period. Samples were collected in 1-l sterile high-density polyethylene sample bottles (Cole-Parmer, Chicago, IL). The locations were municipal water feed, post-reverse osmosis unit, post-mixed-bed deionization tank, post-ultraviolet light sterilizer, and post-sub-micron final filter. Samples obtained in the second month were taken at the post ion exchange bed location both with and without injection of ozonated

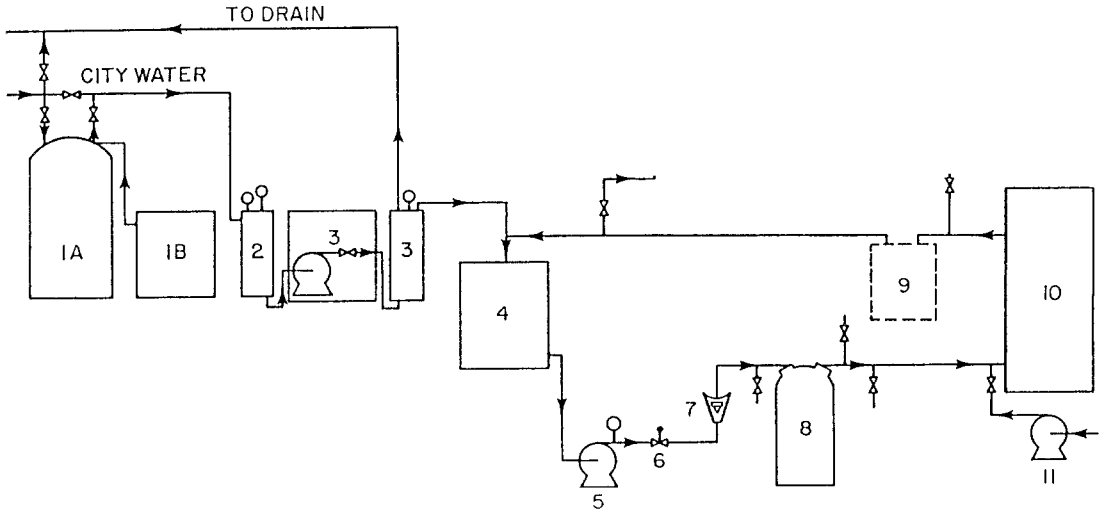

Fig. 1. Schematic diagram of the ultra-pure water system. 1A, water softener; 1B, brine tank; 2, carbon bed; 3, reverse osmosis unit; 4, PVDF storage tank; 5, PVDF circulation pump; 6, PVDF diaphragm valve; 7, PVDF flow meter; 8, mixed bed ion exchange tank; 9, final filter; 10, UV sterilizer; 11, Ozone metering pump.

water (Ozone generator manufactured by OREC, Phoenix, AZ, model O3V5-O) upstream of the sampling point. Samples were also taken from the line leading from the ozone contact tank to the pilot plant. Each sample was then filtered through a 0.2-μm pore-size membrane (Gelman Scientific, Ann Arbor, MI). The filters were then transferred to the agar where they were incubated for 72 h at room temperature (23 °C). Sample volumes ranged from 10 ml to 1 l based on suspected bacterial levels. Equal sample volumes were assayed on both R2A or Tryptic Soy Agar (TSA) (Difco, Detroit, MI).

Calculation of total plate counts and statistical analyses

All data were converted to counts per liter for ease of comparison. The Student's *t*-test [3,4] was used to determine statistical variation and confidence limits of plate counts.

RESULTS AND DISCUSSION

The average bacterial numbers per liter obtained at various sample points throughout the system are shown in Fig. 2. Confidence levels for the data were 90% or better at the city water, post-reverse osmosis, post-ultraviolet sterilizer, and at the ozonated post-ion exchange locations. Confidence levels of 85% were obtained at the post-ion exchange and post-filter locations. Bacterial numbers in the system decreased as a function of treatment steps. The order of decreasing total plate counts can be listed as tap water, post-RO, post-ion exchange bed, post-filter, post-UV, post-ion exchange bed (+50 ppb ozone at outlet of bed), and ozonation tank. Direct count methods such as acridine orange direct count (AODC) cannot be employed on a routine basis because of the low numbers of bacteria found [12]. In this system, the mechanisms of viable cell removal by adsorption through surface charge interactions are dominant for the ion-exchange bed [7]. Size exclusion is probably the dominant mechanism for bacterial removal for the reverse osmosis unit.

An additional exposure of 50 μg per liter ozone in the water decreased the viable bacterial numbers in the system when compared with the non-ozonated water (100 per liter reduced to <10 per liter). Ozonation, therefore, may be considered as an effective killing agent in this system. However, it must be noted that the ozone must be completely destroyed by UV radiation [6,8,9,13], as exposure to ozone will damage downstream process equipment such as ion exchange beds through direct oxidation of the resins [13], as well as semiconductor devices through hydrophobic/hydrophilic interactions [2] and direct oxidation of the wafer surface [5]. R2A medium consistently showed greater bacterial counts when compared with TSA medium.

Fig. 3 shows the effectiveness of each of the system

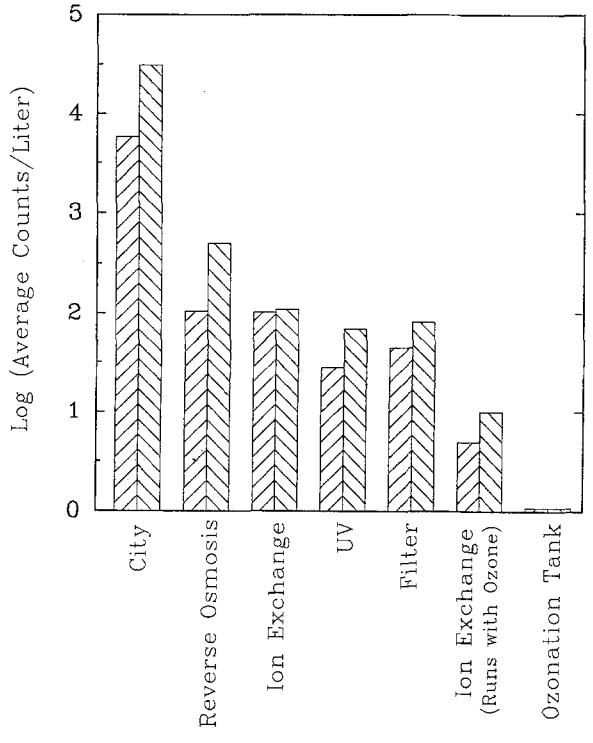

Fig. 2. Bacterial counts in the outlet of system components. ▨, TSA medium; ▧, R2A medium.

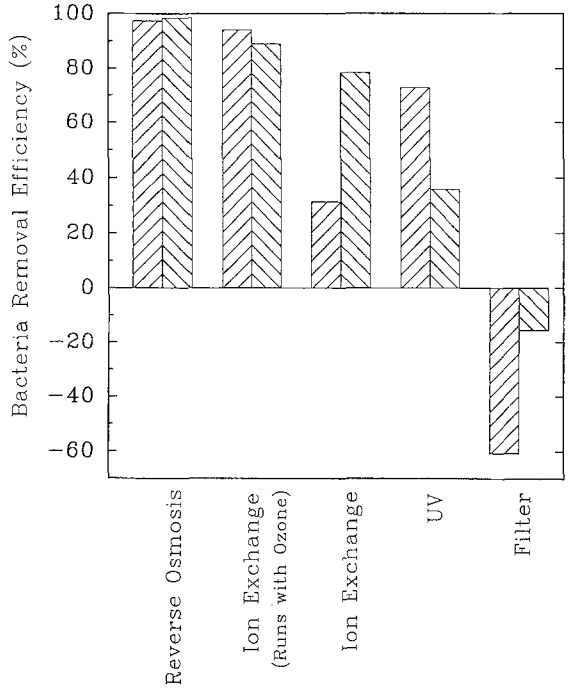

Fig. 3. Bacteria removing effectiveness of system components. ▨, TSA medium; ▧, R2A medium.

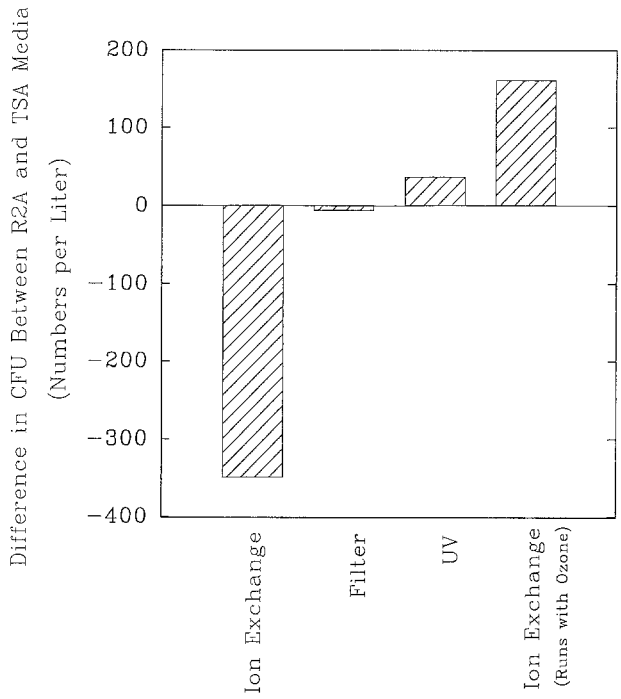

Fig. 4. Difference in cfu between R2A and TSA media in various locations of ultra-pure water treatment system.

components in the removal of bacteria from the ultra-pure water pilot plant. Reverse osmosis demonstrated better than 97% removal while effluent from the final filter showed an increase in numbers. This may be due to the concentration of bacteria and nutrients constantly accumulating on the surface of the filter. Bacteria can then regrow through the filter [7].

Although greater numbers of bacteria were detected on R2A medium in all cases, removal efficiencies were determined to be greater on TSA medium for the reverse osmosis and ion exchange bed treatments than on R2A medium. However, greater removal efficiencies were detected for ultraviolet disinfection on R2A compared with TSA. This indicated that some cells that were damaged under the effects of UV-185 radiation and ozone were not recovered on the TSA medium. The use of R2A may therefore be a better choice of media to obtain a more accurate representation of the number of viable cells after disinfection in the polishing loop of an ultra-pure water system.

The difference in bacteria numbers observed to grow on TSA vs. R2A media is shown in Fig. 4.

This difference can be expressed as:

$$P = (N_{in} - N_{out})_{TSA} - (N_{in} - N_{out})_{R2A}$$

where 'N' is the number of viable bacteria per liter observed for each type of medium. This population value will be greater than zero when the $(N_{out})_{R2A}$ term is large; this indicated that a process unit contains bacteria that may not be able to use TSA medium effectively for growth. The population value will be less than or equal to zero when the $(N_{in})_{R2A}$ term is large; this indicates that fewer TSA-incapable cells are generated which cannot grow on TSA. This population term is important to the water plant since the operators need to know which process units are removing bacteria, and those in which bacteria are capable of growth. The data indicates that the presence of ozone or a UV unit may decrease bacteria, but bacteria can grow on the surface of a filter. If all bacteria are not destroyed, evidence suggests that they can grow and recontaminate the water.

From the analysis of these data, it can be concluded that the use of a non-selective medium capable of supporting oligotrophic bacteria may be necessary in an ultra-pure water system to obtain a more representative estimation of viable cells; the R2A medium can be used for this purpose. The analysis of the system components with respect to the effect on bacteria indicates that the actions of the reverse osmosis unit, ozone ion exchange and UV sterilization aids in the reduction of bacterial numbers. The mechanism of bacteria removal by UV and ozone appears to occur through direct injury of the cells [7]. Size exclusion appears to predominate as the mechanism of removal of cells by the reverse osmosis unit [7]. The mechanism of bacterial removal by the ion exchange bed may be due to selective adsorption of the cells to the resin by cell/resin charge interactions. An increase in the numbers of bacterial cells was observed across the nylon membrane; this indicates that nutrients may concentrate on the surface of the filter, bacteria may use these nutrients to reproduce, and growth of the cells through the filter may occur [7].

ACKNOWLEDGEMENT

This work was supported by Sematech through a contract to the University of Arizona.

REFERENCES

1 Atlas, R. and R. Bartha. 1987. Effects of abiotic factors on microorganisms. In: Environmental Microbiology: Fundamentals and Applications, 2nd edn. (Mautner, S. ed.), pp. 254–255, The Benjamin/Cummings Publishing Company, Menlo Park, CA.

2 Donovan, R., A. Clayton, D. Riley, R. Carbonell and V. Menon. 1990. Investigating particle deposition mechanisms of semiconductor devices exposed to aqueous baths. Microcontamination. 8: 5–29.

3 Green, D. 1984. Conversion factors and miscellaneous

tables. In: Perry's Chemical Engineering Handbook, 6th edn., (Green, D. and J. Maloney, eds.), p. 24. McGraw-Hill Book Company, New York, NY.

4 Hoerl, A., M. Nashed, J. McKetta and I. Silverberg. 1984. Mathematics. In: Parry's Chemical Engineering Handbook, 6th edn., (Green, D. and J. Maloney, eds.), pp. 85–87. McGraw-Hill Book Company, New York, NY.

5 Maly, W. 1987. Processing Steps. In: Atlas of IC Technologies: An Introduction to VLSI Processes, (Bartholomew, C. and M. Westermann, eds.), pp. 8–13. The Benjamin/Cummings Publishing Company, Menlo Park, CA.

6 Meyers, P. 1987. Destruction of TOC using ultraviolet light. Forty-Eight Annual Meeting of the International Water Conference Transcript. 1–22. International Water Conference, Pittsburgh, PA.

7 Mittelman, M. and G. Geesey. 1988. Biological fouling. In: Biological Fouling of Industrial Water Systems: A Problem Solving Approach (Mittelman, M. and G. Geesey, eds.), pp. 1–16, 194–233. Water Micro. Assoc., San Diego, CA.

8 Paleg, M. 1976. The chemistry of ozone in the treatment of water. Water Res. 10: 361–365.

9 Peyton, G., F. Huang, B. Burleson and W. Glaze. 1982. Destruction of pollutants in water with ozone in combination with ultraviolet radiation. 1. General principles and oxidation or tetrachloroethylene. Environ. Sci. Tech. 16: 448–453.

10 Reasoner, D. and E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 50: 1–7.

11 Wilkins, F. and P. McConnelee. 1989. Continuous deionization in the preparation of microelectronics grade water. Sld. St. Technol. 32: 89–92.

12 Yabe, K., Motomura, Y., Ishikawa, H., Mizuniwa, T. and T. Ohmi. 1989. Responding to the future quality of ultra-pure water. Microcontamination. 7: 37–45.

13 Yarnell, P., Kato, M. and B. Hoffman. 1989. The effects of ozone on ion exchange mixed beds in ultra-pure water. 8th Annual Semiconduct. Pure Water Conf. Trans. pp. 195–221. Semiconductor Pure Water, Palo Alto, CA.