

Trickle Irrigation: Predominant Bacteria in Treated Colorado River Water and Biologically Clogged Emitters *

R. G. Gilbert¹, F. S. Nakayama¹, D. A. Bucks¹, O. F. French¹,
K. C. Adamson¹, and R. M. Johnson²

¹ U.S. Water Conservation Laboratory, 4331 East Broadway, Phoenix, AZ 85040, USA

² Arizona State University, Tempe, AZ 85282, USA

Received July 21, 1981

Summary. Bacterial numbers and predominant bacterial types were determined in trickle irrigation systems receiving treated Colorado River water. Fourteen bacterial genera were isolated and identified from 86 water and sediment samples collected from trickle emitter systems receiving six water treatments. The bacteria identified were common aquatic and soil microbes and the genera in order of prevalence were *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Brevibacterium*, *Micrococcus*, and *Bacillus*. A greater diversity of bacterial types was found in water that was sand filtered and received no chemical treatment. Regardless of the water treatment, *Pseudomonas stutzeri* was the predominant bacterium followed by *Flavobacterium lutescens*. The data indicated that pigmented bacteria, *F. lutescens* and *Cytophaga hutchinsonii* caused the yellow color of the slime deposits in biologically clogged emitters; and, their growth was presumably supported by *P. stutzeri*, a nonpigmented bacterium. The occurrence of *Bacillus* sp. was enhanced by sand and screen filtration and markedly reduced by chemical conditioning the water with either chlorine and acid or acid alone. No strictly anaerobic bacteria, such as *Clostridium* sp., were detected in water treated with chlorine and acid. Iron bacteria, *Sphaerotilus* spp., were not detected in any water or sediment samples from trickle irrigation systems.

Introduction

Trickle irrigation is a reliable and efficient method of irrigating high valued row and tree crops. It helps to increase yields and to decrease labor costs and water usage. However, clogging of emitters remains a major problem that adversely affects the water distribution efficiency and the useful life of a system. Emitter clogging usually results from some type of physical, chemical, or biological agent.

* Contribution from the U.S. Department of Agriculture, Science and Education Administration, Agricultural Research

Physical clogging problems have been well documented (Gilbert et al. 1981; Wilson 1972, 1975) and preventative methods of water filtration have been used successfully (Gilbert et al. 1979; Nakayama et al. 1978). Chemical clogging problems due to the precipitation of calcium or magnesium carbonate have been prevented by chemically conditioning the water with acid to control the pH (Nakayama et al. 1977, 1978; Pelleg et al. 1974). Biological clogging problems have been reported (Ford 1975; Ford and Tucker 1974, 1975; McElhoe and Hilton 1974), especially where iron and sulfur bacteria have produced a brownish-orange or white gelatinous slime, respectively (Ford 1979).

The growth and development of other aerobic slime-forming bacteria have caused emitter clogging problems (Ford 1977; McElhoe and Hilton 1974; Sharp 1956). This report describes the predominant bacteria that were present in trickle irrigation systems receiving treated Colorado River water at the end of 4 years of operation. The genera of bacteria that clogged emitters with biological slime deposits were also identified.

Methods and Materials

Background

A field study was established in January 1975 on 0.8 ha with 200 mature Valencia orange trees at Tacna, Arizona, 64 km east of Yuma, Arizona, in the Wellton-Mohawk Irrigation and Drainage District. Details of the experimental site, equipment, chemical water analysis, and operational procedures were described previously (Gilbert et al. 1979; Nakayama et al. 1978).

Types of Emitters

Eight different types of emitters were selected from a representative sampling of emitter designs commercially available (Table 1). The emitters were fitted into 1.3 cm polyethylene tubing and circled symmetrically around each of the four trees per subplot.

Water Treatments

Water treatments (Table 2) were varied by degree of filtration for removal of suspended load and amount of chemical conditioning for controlling pH and preventing biological slime

Table 1. Description of trickle emitters

Emitter	Type	Flow rates ^a L/h	Orifice size ^b mm
1	Long-path, capillary tubing	6.5	0.95
2	Long-path, spiral-grooved, manual flush	7.6	0.95
3	Long-path, spiral-grooved, manual flush	3.8	0.74
4	Diaphragm, expandable orifice	3.4	0.58
5	Short-path, spiral-grooved, removable insert	5.7	0.64
6	Automatic, needle-flush valve	3.8	0.58
7	Automatic, ball-flush valve	4.6	0.27
8	Single vortex	4.2	0.64

^a Initial flow rate of emitter at 104 kPa

^b Orifice size represents the minimum internal diameter of the water flow path

Table 2. Description of water treatments

Treatment	Type of treatment	
	Filtration ^a	Chemical
A	Screen (50-mesh)	None
B	Screen (50-mesh)	Chlorine & acid-intermittent ^b
C	Sand + screen (200-mesh)	None
D	Sand + screen (200-mesh)	Chlorine & acid-intermittent ^b
E	Sand + screen (200-mesh)	Chlorine & acid-continuous ^c
F	Sand + screen (200-mesh)	Acid-continuous

^a The sand filter medium was No. 20 silica sand, and the screens were stainless steel, cylinder-type commercial units

^b Intermittent chemical treatment: Chemicals were injected only during the last hour of irrigation cycle. The residual chlorine concentration was 10 mg/l and the acid adjusted the river water to pH 7. Chemical injection was started after 278 days of operation for Treatment B

^c Continuous chemical treatment: Chemicals were injected during the complete irrigation cycle. The residual chlorine concentration was 1.0 mg/l (Treatment E) and the acid adjusted the river water to pH 7 (Treatments E and F)

development. The water in Treatments A and C received no chemical conditioning. For Treatment E, dilute calcium hypochlorite and sulfuric acid solutions were injected separately into the inlet side of the sand filter. Injection rates of the chemicals were adjusted to attain a pH of 7, and the free residual chlorine in the water at the outlet of the screen filter was about 1.0 mg/l. In Treatments B and D, acid and hypochlorite were injected intermittently only during the last hour of irrigation, so that the treated water filled all lines from the treatment shed to the lateral ends in the field. This produced residual chlorine concentrations of about 10 mg/l and adjusted pH to 7. In Treatment F, dilute sulfuric acid was injected continuously at a rate sufficient to adjust the pH to 7.

Quantitative Bacterial Assays

Water samples for bacterial analysis were collected from Colorado River water and four of the eight emitter systems in sterile plastic bottles (250 ml) at 2- and 3-week intervals in the summer and winter, respectively. Colorado River water samples were obtained in the treatment shed before the water was filtered or chemically conditioned. Emitter water samples were obtained by placing plastic bottles under emitters elevated with a wire support above the soil surface. Since the temperature and water flow through the system would affect the growth and establishment of bacterial populations in the emitters and lateral lines, all emitter water samples for bacterial analysis were collected at the start of the irrigation cycle. Thus we measured maximum diurnal growth responses between the daily irrigation cycles that ranged from about 2 h in the winter to 12 h in the summer. The emitter water samples were collected from the field within 5 to 10 min after the irrigation system was started. Upon returning to the treatment shed, the emitter water samples were quickly inoculated into Millipore³ "Water Testers" (Millipore Corp. 1975). The inoculated water testers were transported from the field to the laboratory and incubated for 7 to 10 days at 28 °C before the total number of bacterial colonies was counted.

The water testers are presterilized, self-contained, disposable filtration and incubation units used for the microbiological examination of water. Each one contains a 0.45 µm

³ Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the author or the U.S. Department of Agriculture

membrane filter backed with an absorbent pad containing dehydrated Tryptone Glucose Yeast Extract medium. When dipped into an aqueous fluid 1.0 ml was automatically absorbed through the membrane filter. The nutrient pad was hydrated providing the culture medium for the bacteria retained on the membrane filter. The water testers were especially useful, since laboratory facilities were not available at the experimental field site and conventional sampling and transportation of samples were not convenient. The use of these water testers served two purposes: 1) to monitor and evaluate how effectively the chlorination treatments controlled microbial growth; and 2) to determine the relative number of heterotrophic aerobic bacteria in the water samples from the varied water treatments and the Colorado River.

Qualitative Bacterial Assays

Qualitative assays of bacterial populations were carried out in March and April of 1979, just before termination of the 4-year experiment. All water samples, trickle emitters, and tubing segments from lateral lines collected at the field site for biological analysis were transported on ice and stored at 4 °C in the laboratory. Water samples were collected from the Colorado River and flowing trickle emitters as previously described. Sediment samples were collected from inside segments of the polyethylene lateral line tubing and from the internal flow channel of trickle emitters. Microbial slime samples were collected from the internal flow channel of emitters that were biologically clogged. Biological samples of water and sediment from each type of trickle emitter and water treatment were assayed for the predominant bacterial genera.

Emitters and tube segments were surface sterilized in 5% sodium hypochlorite and carefully dissected with tools and equipment that had been flame sterilized with 95% ethanol. Sediment samples from longitudinally cut tube segments and dissected emitters were aseptically scraped into 20 ml of sterile water. These samples were transferred into sterile milk dilution bottles and stored at 4 °C, until they were analyzed for bacterial genera. All water and sediment samples were treated with sodium thiosulfate (100 mg/l) to neutralize any residual chlorine.

Identification of Bacteria

Within 24 h after collecting the water and sediment samples, we inoculated them onto four culture media: neopeptone agar (NEO), streptomycin assay agar (SM), desoxycholate agar (DES), and trypticase soy agar (TSA). Streak inoculations were made with 0.5 ml of sample dilutions or 0.1 ml of the undiluted sample on each culture medium. All streaked culture plates were incubated aerobically at 25 °C for 3–5 days and only streaked culture plates of TSA were incubated anaerobically at 25 °C for 5–7 days. Anaerobic incubations were performed with BBL Gas Pack systems³.

The predominant colonies were counted and isolates were selected and subcultured on TSA slants. Each isolate was examined for growth at 37 and 45 °C; for growth on 5 and 10% salt in TSA; for hydrolysis of starch and gelatin; and for fermentation reactions on glucose. Anaerobic growth of each isolate was examined on TSA plates. The oxidase test was done on all cultures using pathotec oxidase strips. The catalase test was observed after applying a 3% hydrogen peroxide solution to a cell mass on a glass slide. Pigmentation was determined primarily on SM and gelatin cultures. Migration ability was observed on 0.5% peptone agar. Fluorescence of cultures growing on King's fluorescent medium was examined under short-wave (254 nm) ultraviolet light. Cell morphology and gram stain reactions were made on isolates cultured for 24 to 48 h. Predominant bacterial types were established by colonial growth prior to generic identification of each bacterial isolate. Classification was done according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974).

Assays for filamentous bacteria, *Sphaerotilus* and *Leptothrix* (iron bacterium), were performed by adding 5.0 ml of each biological sample to flasks containing 25 ml distilled water with 0.05% yeast extract, 0.1% peptone, 0.5% calcium carbonate, 0.01% magnesium sulfate and a piece of broken razor blade as an iron source. These inoculated flasks were incubated in darkness at 25 °C for at least 21 days and occasionally for 6 weeks. Periodically the flasks were examined for filamentous growth and floculations of iron bacteria.

Results and Discussion

Quantitative Assays of Bacteria Populations

Relative numbers of bacteria were determined in trickle irrigation systems receiving treated Colorado River water. Water treatments were varied based on the degree of filtration for removal of suspended load and on the amount of chemical conditioning for controlling pH and preventing biological slime development. Bacterial numbers in Colorado River water (Fig. 1 A) were usually in the 100 to 500 cells/ml range, which indicated the main canal water was comparable to other non-eutrophic water sources. Populations were seasonal – numbers of bacteria were higher in summer than in winter. Filtration of Colorado River water with either screen (50-mesh) filters only or combinations of sand and screen (200-mesh) filters did not alter the number of bacteria in the water (Fig. 1 B). Chemical conditioning of the water either intermittently or continuously with chlorine and acid effectively controlled the growth of bacterial populations and development of biological slime by periodically reducing bacterial numbers (Fig. 1 C, D). Occasionally, the residual chlorine was sufficient to prevent bacterial regrowth between irrigation cycles and bacteria were not detected by the culture methods used. The number of bacteria in water continuously conditioned with acid alone were not markedly changed (Fig. 1 D) as compared with the other treatments.

Qualitative Assays of Bacterial Populations

Fourteen bacterial genera were identified in 86 water and sediment samples collected from trickle emitter systems receiving six water treatments (Table 3). The bacteria present were common aquatic and soil microbes and the most prevalent population types were not markedly changed by the water treatments. The major genera in order of their occurrence were *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Brevibacterium*, *Micrococcus*, and *Bacillus*. *Pseudomonas* spp. occurred in 87% of the biological samples and their prevalence was not changed by filtering and chemically conditioning the water. Water conditioned continuously with chlorine and acid was the only treatment in which *Pseudomonas* sp. shared predominance with *Flavobacterium* sp. The greatest diversity of bacterial isolates, 16 to 20, was present in water and sediment samples that received no chemical conditioning (Treatments A and C). Water conditioned continuously with acid (Treatment F) had six or fewer different isolates. Water intermittently or continuously treated with chlorine and acid (Treatments B and E) had only two to three different isolates.

Among the bacterial isolates, characteristic soil bacteria were isolated, which included the gram positive rods of *Bacillus* sp. and coryneform bacteria, the gram negative genera of *Acinetobacter*, as well as the strict anaerobes *Clostridium* and *Propionibacterium*. *Clostridium* was not isolated as a predominant bacterium from water that was sand and screen filtered and chemically treated. *Vibrio* sp., biochemically similar to *Pseudomonas* sp., were found in all treatments except the continuous acid (Treatment F). The unexpected detection of *Erwinia* occurred in only one sample from water conditioned continuously with acid. *Staphylococcus* spp. were not considered indigenous to Colorado River water; its detection eight

Table 3. Percent of water and sediment samples containing various bacterial genera. The samples were from trickle irrigation systems receiving filtered and chemically treated Colorado River water

Genera of Bacteria	Water Treatments						
	Colorado River Water (Control)	Screen (50-mesh) filtration		Sand and Screen (200-mesh) filtration		Acid (only) Continuous (F) (%)	
		No Chemicals (A) (%)	Chlorine + Acid Intermittent (B) (%)	No Chemicals (C) (%)	Chlorine + Acid Intermittent (D) (%)		Chlorine + Acid Continuous (E) (%)
<i>Pseudomonas</i>	100	83	83	100	57	38	77
<i>Flavobacterium</i>	100	33	33	20	21	46	23
<i>Vibrio</i>	-	42	42	15	29	23	-
<i>Brevibacterium</i>	-	17	17	20	7	8	15
<i>Micrococcus</i>	-	-	17	15	36	15	31
<i>Bacillus</i>	-	25	-	45	-	8	-
<i>Staphylococcus</i>	-	-	8	5	29	8	15
<i>Acinetobacter</i>	-	17	-	5	7	8	15
<i>Clostridium</i>	-	25	8	15	-	-	-
<i>Corynebacterium</i>	50	8	-	10	-	-	15
<i>Cytophaga</i>	-	25	-	5	7	8	-
<i>Arthrobacter</i>	-	-	8	5	-	-	-
<i>Erwinia</i>	-	-	-	-	-	-	8
<i>Propionibacterium</i>	-	-	-	-	-	-	8
No. Samples Assayed	2	12	12	20	14	13	13

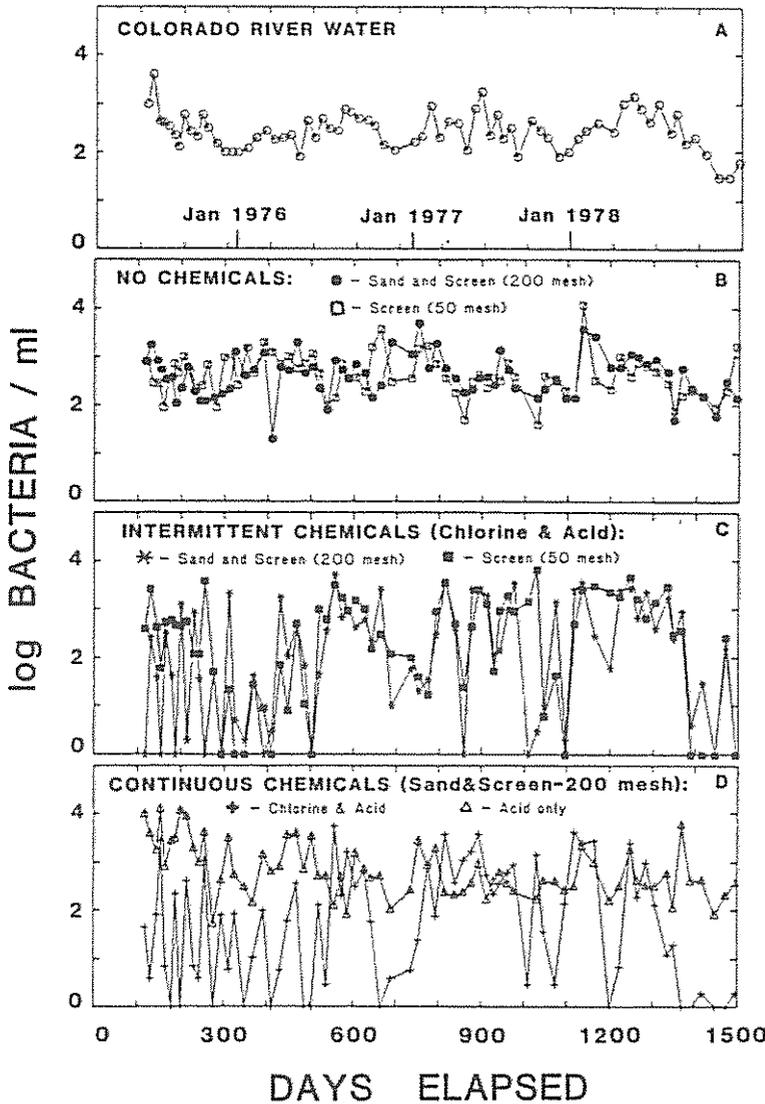


Fig. 1. Effect of filtering and chemical conditioning Colorado River water on relative bacteria numbers in water samples from trickle emitters. (Numbers of 1 or less were plotted as zero.)

times was most likely caused by sample contamination, even through aseptic techniques were used in dissecting and culturing the samples.

The selective culturing procedures used for isolating filamentous bacteria, *Sphaerotilus* and *Leptothrix* (iron bacterium), failed to detect them in any water and sediment samples or biologically clogged emitters. Ford (1977) reported that water containing >0.4 mg/l of ferrous iron contributed to the growth of filamentous iron bacteria and caused emitter clogging problems. Apparently, Colorado River

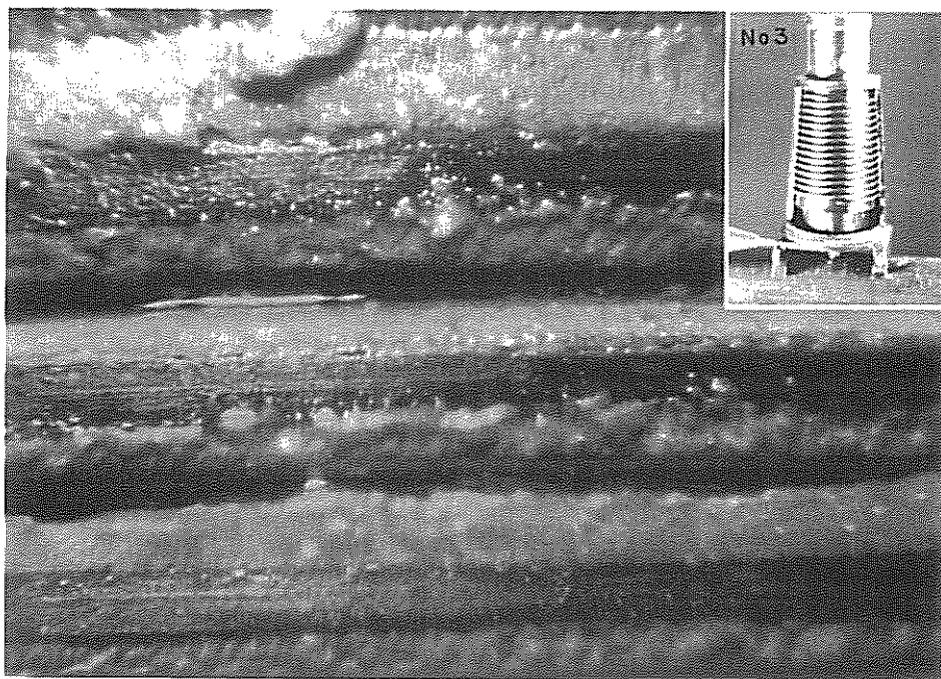


Fig. 2. Illustration of microbial slime deposits that completely clogged the spiral-grooved flow-path of emitter 3 (Mag. 10X). *Pseudomonas stutzeri* and yellow pigmented *Flavobacterium lutescens* were identified as the predominant bacteria (Table 4). The insert (upper right) is a dissected view of emitter 3 attached to a lateral line

Table 4. Percent of biologically clogged emitters containing various bacterial genera. The Emitters were from trickle irrigation systems receiving chemically treated and nontreated Colorado River water^a

Bacteria	Percent Detected (%)
<i>Pseudomonas stutzeri</i>	89
<i>Flavobacterium lutescens</i>	44
<i>Vibrio</i> sp.	22
<i>Micrococcus</i> sp.	22
<i>Brevibacterium linins</i>	22
<i>Acinetobacter calcoaceticum</i>	22
<i>Bacillus</i> sp.	11
<i>Clostridium</i> sp.	11
<i>Cytophaga hutchinsonii</i>	11
<i>Pseudomonas aeruginosa</i>	11
No. of emitters assayed	9

^a Of the emitters assayed for genera of bacteria, seven were from chemically treated and two were from nontreated systems

water which contained <0.1 mg/l of ferrous iron (Gilbert et al. 1979) had not induced the growth and clogging activity of these bacteria in our trickle systems.

Gilbert et al. (1979) reported an unexplained reduction in emitter flow rates with sand and screen (200 mesh) filtration (Treatment C), as compared with only screen (50 mesh) filtration (Treatment A) for emitters 2 and 3 (Table 1); this reduction was prevented by chemically-conditioned water. Since there was no logical physical explanation for the observed flow reductions with Treatment C, biological agents may have caused the problem. These current results have shown that *Bacillus* spp. are not a dominant bacteria in chemically treated water. Therefore, the increase in occurrence of *Bacillus* spp. in untreated water (Table 3) and their association with the dominant *Pseudomonas* spp. and other organisms may be contributing to the flow reduction of emitters 2 and 3 in Treatment C.

The prevalent occurrence of *Pseudomonas* sp. could eventually cause some serious clogging problems. It has been shown that iron accumulates and concentrates in the sediments and organic materials in the main, submain, and lateral lines (Nakayama et al. 1978), and that nonfilamentous aerobic *Pseudomonas* spp. are able to oxidize and precipitate soluble ferrous iron, which clogs emitters (Ford 1977). We have found *Pseudomonas stutzeri* to be the major bacterium present in biologically clogged emitters (Fig. 2) from chemically treated and nontreated trickle systems (Table 4). Along with *P. stutzeri*, the pigmented genera *Flavobacterium* and *Cytophaga*, were the major bacteria in emitter sediment samples. The data suggest that both *Flavobacterium lutescens* and *Cytophaga hutchinsonii* caused the yellow pigmented growth in the emitters. In general, the biological clogging problems with Colorado River water were minimal during the 4 years of operation. The principle clogging problems resulted from physical factors and less than 1% of the 1800 emitters installed demonstrated reduced flow rates or clogging caused by biological slime deposits (Gilbert et al. 1981). From these current results and others (Gilbert et al. 1979, 1981; Nakayama et al. 1977, 1978), water treatments and management practices designed to prevent emitter clogging caused by chemical, physical, and biological agents have been recommended (Bucks et al. 1979).

References

- Buchanan RE, Gibbons NE (1974) *Bergey's Manual of Determinative Bacteriology*. 8th Edition. Williams and Wilkins Publishing Company, Baltimore, MD
- Bucks DA, Nakayama FS, Gilbert RG (1979) Trickle irrigation water quality and preventive maintenance. *Agr Water Manage* 2: 149
- Ford HW (1977) The importance of water quality in drip/trickle irrigation systems. *Proc Int Soc Citricult* 1:84
- Ford HW (1979) Characteristics of slime and ochre in drainage and irrigation systems. *Trans ASAE* 22:1093
- Ford HW, Tucker DPH (1974) Clogging of drip systems from metabolic products of iron and sulfur bacteria. *Second Int Drip Irrig Congr Proc*, San Diego, CA, pp 212-214
- Ford HW, Tucker DPH (1975) Blockage of drip irrigation filters and emitters by iron-sulfur bacterial products. *Hort Sci* 10:62
- Gilbert RG, Nakayama FS, Bucks DA (1979) Trickle irrigation: Prevention of clogging. *Trans ASAE* 22:514
- Gilbert RG, Nakayama FS, Bucks DA, French OF, Adamson KC (1981) Trickle Irrigation: Emitter clogging and other flow problems. *Agr Water Manage* 3:159

- McElhoe BA, Hilton HW (1974) Chemical treatment of drip irrigation water. Second Int Drip Irrig Congr Proc, San Diego, CA, pp 215–220
- Millipore Corp (1975) Samplers for monitoring microorganisms in liquids. Bull. PB 407. Bedford, MA. 5 p.
- Nakayama FS, Bucks DA, French OF (1977) Reclaiming partially clogged trickle emitters. Trans ASAE 20:278
- Nakayama FS, Gilbert RG, Bucks DA (1978) Water treatment in trickle systems. J Irrig Drain Div, ASCE 104:23
- Pelleg D, Lahav N, Goldberg D (1974) Formation of blockages in drip irrigation systems: Their prevention and removal. Secon Int Congr Drip Irrig Proc, San Diego, Ca, pp 203–208
- Sharp RB (1956) The growth of mucus-forming bacteria in drip-feed irrigation lines. J Agr Eng Res 1:83
- Wilson DL (1972) Filtration, filters and water treatment. Third Drip Irrig Seminar Proc, San Diego, CA, pp 17–23
- Wilson DL (1975) Drip irrigation filtration problems and research. Sprinkler Irrig Assoc Proc, Atlanta, GA, pp 51–57