of molecular weight 22 kDa. All isolates had a major protein of MW 43–45 kDa. The specific band (22–24 kDa) was not detected if the isolates were grown on the agar media.

Discussion

Deep groundwater normally has very low heterotrophic and *Aeromonas* counts (Schubert 1991). The presence of *Aeromonas* thus indicates surface water contamination and the existence of an external environment that supports colonization and multiplication of the organism on limestone surfaces. *Aeromonas* spp. need simple sources of nutrients for multiplication. The slightly alkaline pH of the environment, pH 7.1 to 8.1, enhanced the survival of the organism. The CFUs of *A. hydrophila* and the heterotrophic counts decreased significantly following the construction of the new water collection pipeline inside the mine, indicating the importance of surface colonization.

To fully understand the epidemiological links between environmental and clinical aeromonads, both phenotypic and genotypic typing methods must be used to properly fingerprint the strains. This investigation confirmed results reported in some other studies (Havelaar et al. 1992, Moyer et al. 1992). The strains that have been isolated from drinking water are probably rarely similar to those in clinical samples. Biochemical methods and by SDS-PAGE of whole proteins showed that *A. hydrophila* isolated from water resembles genospecies 3, which is uncommon in clinical samples. DNA-DNA hybridization studies confirmed the relatedness of the water strains to the genospecies 3 (results not shown).

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Bacterial Removal over Time in Experimental Up-flow and Down-flow Slow Sand Filters in a Tropical Climate

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Slow sand filters provide water treatment advantages by reducing the amount of chlorine necessary to achieve target disinfection goals and in turn possibly reducing the concentration of chlorination by-products in the treated water. These advantages include 1) removal of organisms from the water before disinfection, 2) removal of turbidity particles, some of which may interfere with disinfection, and 3) reductions in the concentrations of dissolved organic compounds that react with chlorine to form the by-products. This study is designed to determine if satisfactory filtration occurs by using a shallow depth of sand and to investigate the use of up-flow filters, which may be adapted for small water systems in Puerto Rico.

Slow sand filtration is a discontinuous process that depends upon biological activity in the sand to remove microorganisms and organic matter from the water. A normal operational cycle consists of 1) an acclimation period during which the biological activity is established, 2) a long period of steady-state removal of microorganisms from the water, and 3) clogging, which requires interruption of the filtration process for removal of the clogged sand. Once the clogging is eliminated, the operational cycle and another acclimation period are started. To achieve the best overall performance, all phases of the operational cycle must be controlled. Microorganism removal may be limited during the acclimation period and a safe supply of water must be provided during that time. Also, clogging may occur over a short period and interrupt the water supply at an inopportune time.

Small public water systems may lack skilled operation. Thus, the provision of two filters may provide a satisfactory solution to problems presented by the discontinuous nature of the slow sand filtration process. One filter may be acclimated while the other is used for supplying properly treated water. If the filtration rate can be doubled without decreasing the microorganism removal during the steady-state phase, using two filters alternately may not require any greater amount of filter area than a single normal rate filter.

Increasing the filtration rate could result in shorter periods of steady-state removal before clogging occurs. This may be overcome, at least partially, by up-flow operation of filters that are hydraulically stratified. The large sand grains on the bottom of the stratified bed provide larger spaces for the biological growth that is responsible for removing microorganisms and other particles from the source water.

Previous studies of slow sand filtration have failed to provide detailed information on the nature and length of the acclimation period. Up-flow operation of slow sand filters has not been investigated to any great extent, either. Information is being sought on all three operational phases, on the relationship between the filtration rate and the length of the steady state removal phase, and on the feasibility of up-flow operation. This report is concerned with studies conducted during 1991 and 1992.

Experimental slow sand filters were constructed using a 38 to 50 cm depth of sand in an 86 cm glass chromatography column with an inside diameter of 5 cm. The filters have been operated in a water treatment plan in San Germán, Puerto Rico, for more than a year, using the source water for that plant. A diagram of the experimental setup is presented in Figure 1.

The time necessary for acclimation has been observed to be four to five weeks for either mode (up-flow and down-flow) of operation. As shown in Figure 2, locating the transition between acclimation and steady state phases was uncertain because bacterial removal was initially 10% to 20% and gradually increased to more than 95%. Removal of 90% was used as the transition point. Because the operational cycle is so long, only three observations on the length of the acclimation period have been made.

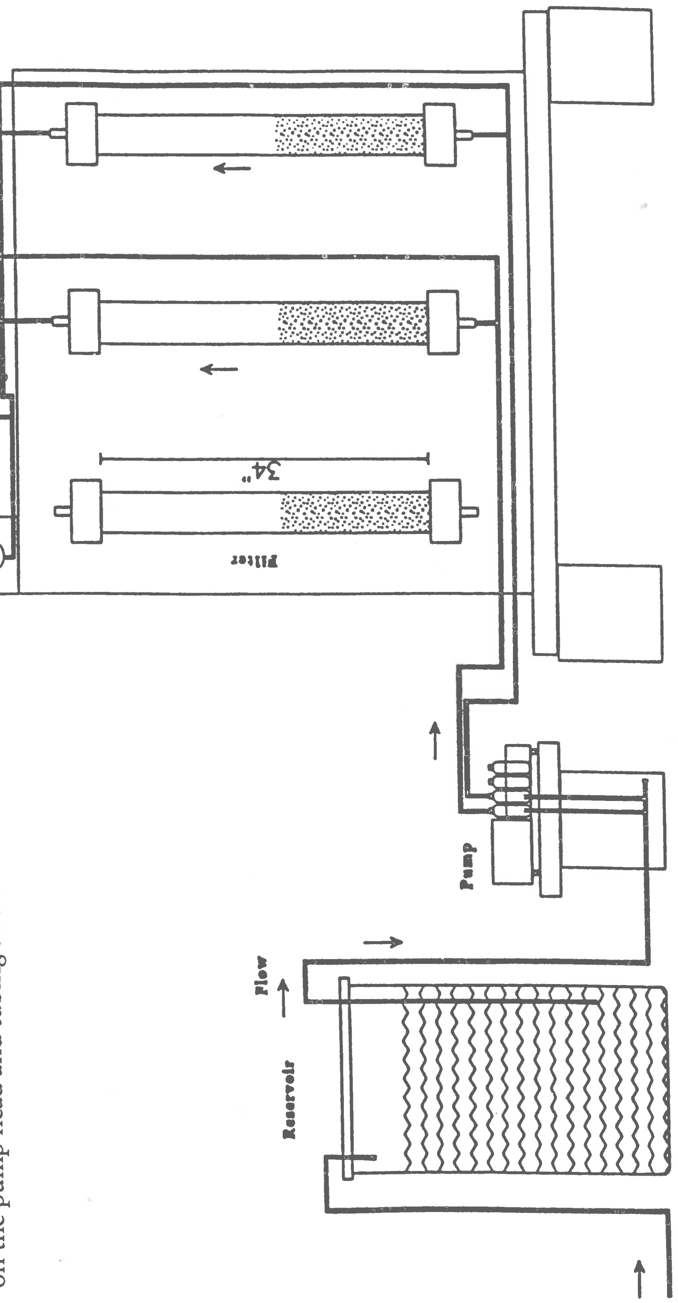

Figure 1.
Schematic diagram of the experimental apparatus. The set-up shown is for two upflow filters. The pump is peristaltic and can deliver 0.001 mL/min to 480 mL/min depending on the pump head and tubing size used.

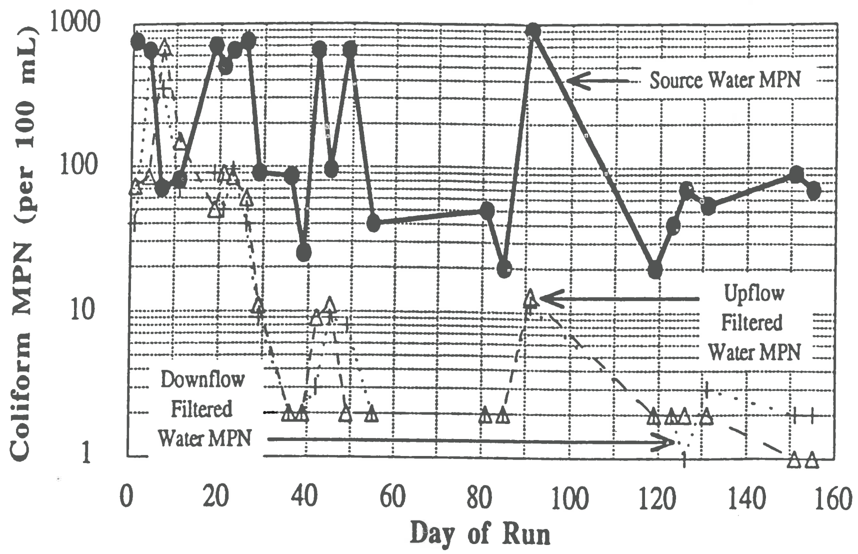

Figure 2. Coliform reductions by up-flow and down-flow filters with approach velocities of 0.4 m/hr (20 mL/min) during the second run.

Due to the effect of rainstorms on water quality, the coliform density of the source water varied between 20 and 900 MPN per 100 mL during the experimental period. Nevertheless, reductions were consistently above 90% during the steady state operational phase. At a flow rate of 10 mL/min (approach velocity = 0.2 m/hr), an up-flow filter reduced the average coliform density (measured by the MPN) by 94%. Figure 2 shows that at a flow rate of 20 mL/min, the up-flow filter reduced the coliform density by 96% (mean filtered water MPN = 5.5 per 100 mL) and the down-flow filter reduced the coliform density by 97% (mean filtered water MPN = 4.4 per 100 mL). Reductions in the plate counts of heterotrophic bacteria were approximately the same as the reductions in coliform density.

These filters, both up-flow and down-flow, were operated for more than 6 months before clogging required that the sand be cleaned. The period of steady state operation appeared to be longer for up-flow filters; however, additional data are needed before this can be considered a firm conclusion.

Normal depth slow sand filters have 90 cm or more of sand at the start of an operational period. When clogging occurs, they are cleaned by removing the top few centimeters of sand for separate washing. These experimental filters are cleaned by back-washing that produces hydraulic stratification of the sand. Following back-washing, chlorination of the sand is used to eliminate the microorganisms before the start of another acclimation period. This procedure, however, would not be a normal practice for operational filters, and shorter acclimation periods may be possible.

The results indicate that slow sand filters are suitable for use in small water systems in tropical climates such as Puerto Rico. Significant reductions in the bacterial content of the source water can be achieved so that the amount of chlorine needed for disinfection can be greatly reduced. Up-flow operation achieves bacterial reductions equivalent to those filters operated in the down-flow mode.

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