Microbial Purification in Slow Sand Filter

AMAL K. DATTA* and MALAY CHAUDHURI†

Environmental Engineering Laboratory,
Department of Civil Engineering,
Indian Institute of Technology,
Kanpur 208016, India

ABSTRACT

Laboratory slow sand filter was characterized in terms of variation in catalase activity and standard plate count with depth as well as its effectiveness in inactivation of enteric microorganisms, using *Escherichia coli* as a model. The term inactivation was used to describe the overall process of removal/inactivation of enteric microorganisms in the filter, including predation, antagonism, etc. The following equation was proposed and used to estimate the inactivation potential of various filter bed depths from data on decay of *E. coli* in reactors with sand microorganism suspension from corresponding depths: $dN/dt = -kN - (pG)N$, in which N is the population of *E. coli* in reactor at time t, k is the decay rate of *E. coli* in control reactor, p is the inactivation potential, and G is the weight of sand sample corresponding to the sand microorganism suspension inoculum. Efforts were also made to examine the relative role of procaryotes and eucaryotes in the inactivation. It was observed that a matured filter bed was well populated throughout with active microorganisms, with its maximum in the top 10 or 25-cm layer. Distribution of the population was independent of the rate of filtration. In general, the top 10 or 25 cm of the filter bed supported a microbial population that contributed most significantly to inactivation of *E. coli.*; the entire bed, however, was active in such inactivation. Both procaryotes and eucaryotes were active in the filter, with procaryotes more dominant near the top surface.

INTRODUCTION

In slow sand filtration, removal of impurities from the raw water is brought about by a combination of many different processes or mechanisms. Possible mechanisms by which a slow sand filter may remove suspended as well as colloidal impurities have been examined by many

* Present address: Department of Civil Engineering, Bengal Engineering College, Howrah 711103, West Bengal, India.
† To whom correspondence should be addressed.

workers. A brief summary of such mechanisms, which include strain-ing, sedimentation, electrostatic attraction, biological oxidation, etc., has been given by Huisman et al. (1981). But such mechanisms fail to account for the most significant purification effect of a slow sand filter, viz., removal/inactivation of enteric microorganisms. Sterile sand beds have been found to be incapable of removal/inactivation of enteric microorganisms when operated at normal filtration rates, indicating that biological activity, resulting from maturation of the bed, is essen-tially responsible for their removal/inactivation.

A possible purification mechanism for enteric microorganisms in a matured slow sand filter is their "attachment" onto the sand grains, followed by "inactivation" within the bed, presumably as a result of biological activity of the indigenous microbial population of the bed. Development of indigenous sand microorganisms is apparently a "phys-iological" process, and attachment of enteric microorganisms onto the sand grains is facilitated by the slimy layer of the sand microorganisms on the grain surface. But the exact role(s) played by the indigenous sand microorganisms in removal/inactivation of enteric microorganisms is not known. However, some eucaryotes known to be predators to bacteria as well as other microorganisms and are capable of producing sub-stances toxic to enteric bacteria, have been found to be present in slow sand filter (Lloyd, 1973; Huisman and Wood, 1974). Removal of viruses in slow sand filtration appeared to be a biological process to Poynter and Slade (1977); however, McConnell et al. (1984) raised doubts about the same but failed to delineate the process of viral inactivation. Re-moval/inactivation of enteric microorganisms in slow sand filter is plau-sibly very complex and involves physicochemical, physiological, as well as biological processes.

The present study investigated microbial purification effects in slow sand filter, using a general term "inactivation" to describe the overall process of removal/inactivation of enteric microorganisms in the filter bed in presence of the indigenous sand microorganisms; the process may include mechanisms like predation, antagonism, etc. The study was undertaken with a simplifying assumption that each layer of the slow sand filter bed has its own potential for such inactivation, and this is due to specific environmental condition and biological activ-ity of the microbial population in a particular layer. Experiments were designed to estimate this inactivation potential at various filter bed depths under differing operating conditions (raw water turbidity and rate of filtration). Efforts were also made to examine the relative role of procaryotes and eucaryotes in such inactivation. *Escherichia coli* was used as a model enteric microorganism. Various depths of the filter bed

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MATERIALS AND METHODS

Experimental Program

Five sets of filter run (filtration experiment) were conducted under five different operating conditions (low turbidity raw water at 0.075, 0.1, 0.2 and 0.6 m/h; high turbidity raw water at 0.1 m/h), employing three identical laboratory filters for each condition. All filters were routinely monitored for head loss and effluent quality (turbidity, total coliforms, and standard plate count). Filter runs were usually terminated after about three weeks of operation, allowing sufficient time for development of indigenous sand microorganisms in the bed. However, the filters operating at 0.6 m/h (low turbidity) and 0.1 m/h (high turbidity) were terminated after two weeks because of excessive head loss. Following termination of a filter run, the supernatant water in the filter column was drained off quickly and sand samples from various filter bed depths were collected through the sampling ports.

Raw Water

Low turbidity raw water was prepared by spiking untreated water, derived from a deep tube well, with settled municipal sewage (50:1) and suspension of clay obtained from the bottom deposits of an irrigation canal (Lower Ganga canal). The water had the following characteristics: pH 8.3–9.2, turbidity 3–6 nephelometric turbidity units (NTU), chemical oxygen demand 2.7–6.8 mg/L, total coliform count (MacConkey agar) 100–8,000 colony-forming units per milliliter (CFU/mL), standard plate count 8.5×10^4–3.2×10^5 CFU/mL, conductivity 825–1,010 μS/cm, and dissolved oxygen 6.0–6.6 mg/L. High turbidity raw water was settled Lower Ganga canal water having the following characteristics: pH 7.5–8.5, turbidity 15–60 NTU, total organic carbon 0.3–2.8 mg/L, total coliform count (MacConkey agar) 34–160 CFU/mL, standard plate count 3.5×10^2–7.5×10^3 CFU/mL, and conductivity 120–150 μS/cm.

Filter and Filter Media

Laboratory slow sand filters were 37.5 mm ID and 1.8 m long perspex tubes with sand depth of 1.0 m over a 0.2-m gravel layer and a supernatant water depth of 0.5 m. Stoppered 10-mm sampling ports at filter

bed depths of 0.0, 0.1, 0.25, 0.5, 0.75, and 1.0 m were provided for collection of sand samples. An additional port at 0.025 m was provided for three filters employing high-turbidity raw water. Effluent glass tubes were extended from the bottom of the filters to well above the sand surface to prevent negative pressure in the bed. Filter sand was prepared from construction grade sand after sieve analysis and necessary modification to yield an effective size of 0.2 mm and uniformity coefficient of 2.5. The filter bed porosity was 0.37.

Collection of Sand Samples

Following termination of a filter run, the supernatant water in the filter was drained off quickly through a bypass port at 100 mm above the sand surface. Rubber stoppers of the sampling ports were removed and sand samples were collected in sterile sampling tubes in two sets; one set was used exclusively for catalase activity estimation, and the other for standard plate count and inactivation potential estimation. Instead of replicate sand samples from a particular filter bed depth, it was thought appropriate to use one sample from the same depth of three filters under identical operating conditions. The volume of work involved for simultaneous processing and analyses of sand samples also necessitated such a protocol. For standard plate count and inactivation potential estimation, sand microorganism suspensions were prepared by vigorously shaking the sand samples in 25-mL sterile tap water.

Standard Plate Count and Catalase Activity

Relative abundance of microorganisms at various filter bed depths was estimated in terms of standard plate count and catalase activity. Standard plate count was performed according to the American Public Health Association (1980) using the sand microorganism suspensions and is reported as CFU/g sand sample. Catalase activity was thought to be more realistic for characterization of the sand samples from various depths in terms of the activity of the microbial population as it alleviates many of the limitations inherent to the standard plate count technique. Catalase activity was estimated by incubating the sand samples in 25-mL sterile tap water for 1 h with a substrate concentration of 100 μmoles of hydrogen peroxide, followed by termination of the reaction by addition of 5 mL of 2 N sulfuric acid. The reaction mixture was then filtered (no. 42 Whatman filter) and the residual hydrogen peroxide titrated with 0.01 N potassium permanganate. Catalase activity is expressed as μmole hydrogen peroxide decomposed/g sand sample/h.

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Inactivation Potential

From the point of view of microbiological quality of the filter effluent, data regarding relative abundance of microorganisms at various filter bed depths are of limited usefulness unless it is demonstrated that the filter bed is also active in removal/inactivation of enteric microorganisms. As such, attempts were made to characterize various depths of the filter bed in regard to their effectiveness in removal/inactivation of enteric microorganisms. Such effectiveness was evaluated in terms of a parameter called "inactivation potential," using E. coli as a model enteric microorganism. The test was carried out in a series of conical flasks (reactors). One hundred milliliters portions of sterile raw water in such reactors, one for each depth of the filter bed sampled, were inoculated with 2 mL of sand microorganism suspension and 1 mL of a washed early log phase suspension (ca. 10^6 CFU/mL) of E. coli (obtained from the Indian Type Culture Association, National Chemical Laboratory, Pune, India). Decay of E. coli in the reactors was monitored for two subsequent days using MacConkey agar pour plates. MacConkey agar was used by McCambridge and McMeekin (1979, 1980) for enumeration of E. coli both by the pour plate as well as membrane filter technique. In MacConkey agar, the E. coli colonies are magneta in color and are more easily detected compared to those in eosin methylene blue agar pour plates. A parallel identical set of reactors was also prepared, each having 500 mg/L actidione (HiMedia Laboratories Private Limited, Bombay, India). Two control reactors, one with and the other without actidione, were also run without sand microorganism suspension. Actidione is known to be active against eucaryotes like protozoa, fungi, and yeasts, but is tolerated at least up to a concentration of 500 mg/L by most bacteria including E. coli (McCambridge and McMeekin, 1979). Hence, the reactors with actidione showed the effect of procaryotes along with bacteriophages, if any, on E. coli inactivation.

The following equation was proposed to estimate the inactivation potential of various filter bed depths from the data on decay of E. coli in the reactors with sand microorganism suspension from corresponding depths:

$$dN/dt = -kN - (pG)N$$

in which N is the population of E. coli in reactor at time t (CFU/mL), k is the decay rate of E. coli in control reactor (per day), p is the inactivation potential (decay rate of E. coli in reactor over and above the normal decay rate) (per gram sand per day), and G is the weight of sand sample corresponding to the sand microorganism suspension inoculum in reactor (gram).

The above equation was integrated to yield the following form, which was used for estimation of inactivation potential (p):

$$\ln (N/N_0) = -(k + pG)t = -k't$$

in which N_0 is the initial population of $E.\ coli$ in reactor (CFU/mL) and k' is the $(k + pG)$, which is the gross rate of decay of $E.\ coli$ in reactor (per day).

The data from the control reactors were used to estimate the value of k while that from the reactors with sand microorganism suspension were used to estimate the inactivation potential (p) of various filter bed depths.

RESULTS AND DISCUSSION

Catalase Activity and Standard Plate Count

In the laboratory filters, both catalase activity and standard plate count were highest at the top surface layer of the filter bed and declined gradually with depth, showing fairly uniform values below a depth of about 10 or 25 cm in most cases. However, while catalase activity decreased by a factor of about 5–10, standard plate count dropped by a factor of about two log. It gave an indication that standard plate count data did not truly reflect the microbial activity of the filter bed. Figure 1 shows the variation in catalase activity and standard plate count with depth in two typical laboratory filters (low and high turbidity at 0.1 m/h).

To account for variation in catalase activity with depth and to facilitate comparison of this variation among filters of different sets, catalase activity ratio (catalase activity at indicated depth/catalase activity at the top surface layer) at various filter bed depths was calculated and averaged for each set comprising three filters. Catalase activity ratio vs depth curves for five sets of filter run are presented in Fig. 2, which indicate presence of active microbial population throughout the filter bed depth, irrespective of the rate of filtration or raw water turbidity, the top 10 or 25 cm being the most active layer.

Inactivation Potential

Inactivation potential at the top surface layer of the filter bed was observed to be maximum in all filters. However, it varied from filter to filter and even among the filters of a set operating under identical operating conditions. As such, to account for the variation in inactivation potential with depth and to facilitate comparison of this variation
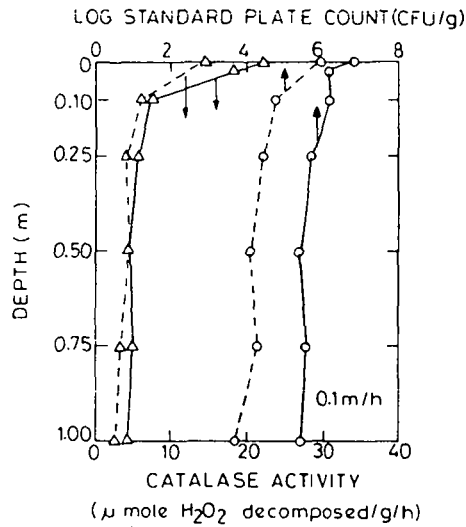
Fig. 1. Catalase activity and standard plate count in two typical laboratory filters (low turbidity: ---; high turbidity: —).

among filters of different sets, inactivation potential ratio (inactivation potential at indicated depth/inactivation potential at the top surface layer) at various filter bed depths was calculated and averaged for each set comprising three filters. Inactivation potential ratio (with and without actidione) vs depth curves for five sets of filter run are also included in Fig. 2. Inactivation potential ratio curves with actidione represent the contribution of actidione-resistant microorganisms (procaryotes).

A fairly distinct picture emerges from Fig. 2 in terms of variation in inactivation potential with depth. Except for the curves for the filter run set using high-turbidity raw water (Fig. 2e), all other curves show a similar trend and clearly indicate that, irrespective of the rate of filtration, inactivation potential below a filter bed depth of about 25 cm did not exceed 20% of that at the top surface layer. Also, variation of inactivation potential with depth almost parallels that of catalase activity. This implies that the top 25 cm of the filter bed harbored a microbial population, which contributed significantly to inactivation of enteric microorganisms, with the entire bed, however, active in such inactivation.

Noticeable difference in variation of inactivation potential with depth (about 50% below a depth of 50 cm) was observed in the filter run set using high-turbidity raw water (Fig. 2e), in spite of a pattern of
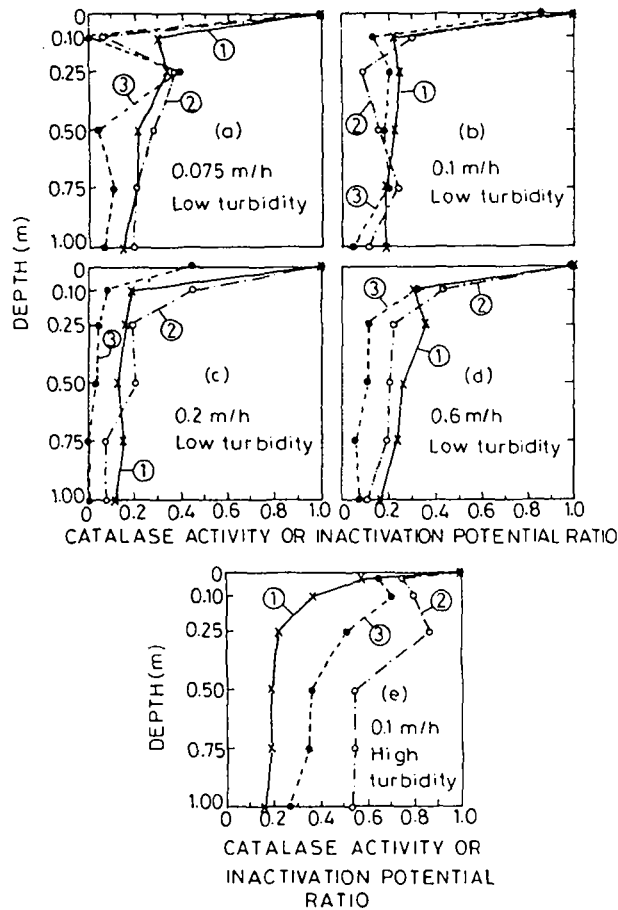
Fig. 2. Catalase activity and inactivation potential ratio vs depth curves. (1) Catalase activity ratio (×), (2) Inactivation potential ratio (○), and (3) Inactivation potential ratio with actidione (●).

decreased catalase activity with depth (about 20% below a depth of 25 cm) similar to that in the low-turbidity sets. Higher inactivation potential of the deeper layers may be accounted for in terms of difference in the source of microorganisms in the raw waters (settled canal water compared to clay and sewage spiked well water). However, poor removal of turbidity and coliforms in all the three filters in this set (Datta, 1987) suggested that higher inactivation potential of the deeper layers of the filter bed was not effective, probably due to high turbidity.

Relative Role of Procaryotes and Eucaryotes

Based on the information presented in Fig. 2, it may be stated that both procaryotes (actidione resistant) and eucaryotes (actidione sensitive) were active throughout the filter bed in activation of enteric microorganisms; however, procaryotes were more dominant in the layer near the top surface of the filter bed.

CONCLUSION

The findings of the present study do not call for any modification in the design and operation of slow sand filter. However, the study provides some additional background on the microbial aspects of slow sand filter. According to the findings, slow sand filter bed harbors a microbial population that is capable of inactivating enteric microorganisms throughout the filter bed, with top 10 or 25 cm of the bed the most active layer. Both procaryotes and eucaryotes are active in the filter bed, procaryotes being more dominant in the layer near the top surface.

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