Removal of Enteroviruses from Sewage by Bench-Scale Rotary-Tube Trickling Filters

NORMAN A. CLARKE* AND SHIH LU CHANG
Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268

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The efficacy of a rotary-tube type of trickling filter for removing coxsackievirus A9, poliovirus 1, and echovirus 12 suspended in raw settled sewage was investigated. At filtration rates equivalent to about 10 MGD (million gallons per day)/acre (ca. 3,785 m³/day per acre), the filters removed 95% of the poliovirus, 83% of echovirus 12, and 94% of coxsackievirus A9. Coliform, fecal streptococci, biochemical oxygen demand, and chemical oxygen demand reductions were remarkably similar, averaging 94, 92, 93, and 95%, respectively. At filtration rates equivalent to about 23 MGD/acre, 59% of the poliovirus, 63% of the echovirus 23, and 81% of the coxsackievirus A9 were removed. Coliform, fecal streptococci, biochemical oxygen demand, and chemical oxygen demand reductions at this filtration rate were 68, 75, 72, and 56%, respectively. Viruses were assumed to be adsorbed to the biological slime growing in the filters, but attempts to disassociate the viruses from the slime were unsuccessful, indicating that the slime-virus complex is very stable or that the viruses were somehow inactivated. The data indicate that coliform and fecal streptococci reductions in this type of sewage treatment process can be used as an index of virus reduction. Disinfection, however, must be used to ensure a virus-free final effluent.

It is now well-documented that enteroviruses are present in raw sewage and that primary and secondary treatment processes vary greatly in their virus-removing capacity. Clarke et al. (6), in laboratory studies, reported that primary sedimentation removed essentially no poliovirus with settling times of up to 3 h, although 50% of the suspended solids had settled. Malherbe and Strickland-Cholmley (13) reported relatively small or no virus removal in field studies on the efficacy of primary sedimentation in removing viruses from raw sewage. However, in their study the virus contained in gross particulate matter (which should be removed by primary sedimentation) was apparently not included in their quantitation of virus. In contrast, however, secondary treatment by the activated sludge process appears quite effective in removing viruses from sewage. In laboratory studies, Clarke et al. (6) demonstrated that in 45 min better than 99% of coxsackievirus A9 was removed in batch tests conducted in bottles, and that in experiments carried out with a bench-model, continuous-flow, activated sludge unit coxsackievirus A9 was reduced about 98% and poliovirus 1 about 90%. Retention times in the unit averaged 7 h, and volatile solids varied from 400 to 4,000 mg/liter; coliform and fecal streptococci removals were greater than 95%.

The efficacy of the activated sludge process in removing viruses from sewage under field or operating plant conditions has been studied by several workers (4, 9, 10, 12). In general, the data indicated that the activated sludge process is quite effective in removing viruses from sewage, although removals are sometimes erratic. These data were usually obtained by comparing the number of positive virus samples after activated sludge treatment with the numbers obtained in the raw sewage. However, as Berg (2) pointed out, the validity of this measuring technique, although seemingly legitimate, may be questioned because of our present inability to accurately quantitate viruses in waste water under field conditions.

The value of trickling filters in removing viruses from sewage has been studied only by a few workers. Kelly and Sanderson (10) reported that plaque counts were reduced about 40% in trickling filter effluents but that viruses were isolated as frequently in the trickling filter effluent as in raw sewage. Isherwood (9) reported that in studies with coxsackievirus A13, which had been added to a small-scale sewage treatment plant, trickling filters appeared to remove less of this virus than did activated
sludge treatment. His results were based upon data from mouse survival experiments; in two experiments 100% of mice inoculated with the trickling filter effluent died with symptoms of coxsackievirus infection, and in a third experiment 25% died. Mouse mortality in four experiments in which the final effluent from the activated sludge system was tested was 12, 12, 0, and 0%, respectively. As Isherwood points out, however, the amount of virus added to his treatment plant was sufficiently large so that even a small fraction passing the trickling filters could cause death in the test animals and, conversely, survival of some of the animals suggests extensive removal of the virus. Malherbe and Strickland-Cholmley (13) have also presented data suggesting that virus removals by trickling filters are not consistent nor of a large magnitude. From all of these findings and the fact that we could find no quantitative laboratory experiments on the efficacy of trickling filters for removing viruses from sewage, the present study was undertaken.

MATERIALS AND METHODS

Viruses. Three enteroviruses were used in these studies: strains of coxsackievirus A9, poliovirus 1, and echovirus 12. The coxsackievirus, designated CME 456, and the attenuated poliovirus 1 (Mahoney), were the same strains used in our previous studies with activated sludge (6). The echovirus 12 (Travis) was a stock laboratory strain that had been plaque purified three times and was in its eighth monkey kidney passage.

Trickling filters. A rotary-tube type trickling filter, as designed by Gloyna et al. (7), was used in these experiments. The three filters were lucite tubes (24 inch [ca. 61 cm] long and 2¼ inch [ca. 5.7 cm] inside diameter). The smooth surface inside of each tube was abraded to allow more even wetting of the surface and to provide a rougher surface on which biological "slime" could grow. Raw settled sewage was fed into the tubes as they were rotated at 16 rpm by means of three individually controlled Brittingham (14) pumps. The sewage feed was held in 55-liter capacity polyethylene containers and stirred with an electric motor-driven paddle at a rate just sufficient to prevent settling of fine particulate matter. Fresh sewage was added to the containers twice daily. Sewage feed rates were varied to provide the equivalent of 8 to 11.5 or 19.5 to 26.0 MGD (million gallons per day)/acre, using the data of Gloyna et al. (7). Gloyna states that "one of the rotating tubes operating at a dosage rate of 7 liters per day of settled domestic sewage has an irrigated wetted surface and flow-through equivalent of a 6-ft-deep bed of 2-inch stone loaded at a rate of 8 MGD." Virus was fed to the rotary tubes by means of an electrolytic pump similar to the one described by Symons (15). Virus was not mixed with the sewage feed, but was pumped directly into the "feed end" of the rotary tube. At the medium filtration rate, sewage was fed at approximately 300 ml/h and virus at 15 ml/h; at the high filtration rate, sewage was fed at about 850 ml/h and virus at 45 ml/h. Details of the entire system, are illustrated in Fig. 1.

Sewage feed. Settled domestic sewage was used as the basic feed in all experiments. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of this sewage were quite low, and therefore the sewage was routinely fortified with ground, emulsified fish food as described by M. C. Mulbarger and J. A. Castelli (21st Annu. Purdue Ind. Waste Conf., Purdue University, Purdue, Ind., 1986). The BOD and COD of the fortified sewage averaged 230 and 345 mg/liter, respectively.

Virus feed. Stock virus suspensions were diluted in Hanks balanced salt solution so that titers averaged 4 x 10^6 plaque-forming units (PFU)/ml for poliovirus, 7 x 10^5 PFU/ml for echovirus 12, and 8 x 10^6 PFU/ml for coxsackievirus. The virus feed was held at 2 to 5°C, and the titers were found to remain stable during the longest experiments, 200 h.

Virus titrations. All virus titrations were conducted using the plaque technique. African green monkey (Cercopithecus aethiops sabaeus) kidney cell cultures were grown in 6-ounce (ca. 180-ml) bottles, essentially by the technique of Huising and Melnick (8). Four- or tenfold dilutions of the suspension being titrated were diluted in Hanks balanced salt solution containing penicillin, dihydrostreptomycin, nystatin, and tetracycline, and each of two plaque bottles were inoculated with 0.5 ml of each dilution. Effluent samples from the trickling filters were allowed to settle for 1 to 2 h at room temperature before virus titrations were conducted on the top third of the supernate. (In normal "field" operation, effluents from trickling filters are settled for about 2 h before discharge.) Inoculated cell sheets were overlaid with approximately 20 ml of agar base medium and incubated at 35 to 37°C. Plaques were counted on the first day they became visible and for 2 or 3 days thereafter.

Coliform and fecal streptococcal counts. Coliform organisms were enumerated by the membrane filter technique using m-Endo broth MP as described in Standard Methods for the Examination of Water and Waste Water (1). Fecal streptococcal counts were made using the membrane filter technique of Kenner et al. (11).

RESULTS

Table 1 presents the results of experiments in which the experimental filters were operated at medium rates—rates equivalent to a filtration rate of about 10 MGD/acre. These data show that the filters removed essentially the same percentage (85 and 83) of poliovirus 1 and echovirus 12. Coxsackievirus A9 removals were higher, averaging 94%. Coliform, fecal streptococci, BOD, and COD removals were remarkably similar, averaging 94, 92, 93, and 95%, respectively.
**TABLE 1. Performance characteristics of rotary-tube trickling filters at medium filtration rates**

| Virus              | Expt | Equivalent* filtration rate (MGD/acre) | Virus reduction (%) | Coliform reduction (%) | Fecal streptococcus reduction (%) | BOD reduction (%) | COD reduction (%) |
|-------------------|------|----------------------------------------|--------------------|------------------------|-----------------------------------|-------------------|------------------|
| Poliovirus 1      | 1    | 10.0                                   | 94                 | 94                     | 97                                | 97                | 93               |
|                   | 2    | 11.5                                   | 84                 | 91*                    | 72                                | 89                | 93               |
|                   | 3    | 11.0                                   | 77                 | 93                     | 88                                | 89                | 96               |
|                   | 4    | 10.5                                   | 84                 | 94                     | 95                                | 92                | 93               |
|                   | X    | 10.8                                   | 85                 | 94                     | 88                                | 92                | 94               |
| Echovirus 12      | 1    | 10.5                                   | 87                 | 97                     | 94                                | 96                | 94               |
|                   | 2    | 8.5                                    | 85                 | 96                     | 98                                | 96                | 94               |
|                   | 3    | 10.5                                   | 65                 | 94                     | 92                                | 91                | 97               |
|                   | 4    | 8.0                                    | 93                 | 91                     | 98                                | 92                | 93               |
|                   | X    | 9.4                                    | 83                 | 95                     | 96                                | 94                | 95               |
| Coxsackievirus A9 | 1    | 9.0                                    | 92                 | 92                     | 97                                | 98                | 94               |
|                   | 2    | 10.0                                   | 87                 | 92*                   | 85                                | 94                | 94               |
|                   | 3    | 10.0                                   | 99                 | 93                     | 92                                | 89                | 98               |
|                   | 4    | 9.5                                    | 96                 | 96                     | 89                                | 91                | 93               |
|                   | X    | 9.6                                    | 94                 | 94                     | 91                                | 93                | 95               |

* Six-foot (ca. 180 cm)-deep bed of 2-inch (ca. 5.1 cm) stone.

* Estimated missing values.
Table 2 presents the results of experiments in which the experimental filters were operated at high rates—rates equivalent to a filtration rate of approximately 23 MGD/acre. Again, these data show that the filters removed approximately the same percentage of poliovirus and echovirus 12, 59% and 63% respectively, but coxsackievirus A9 removal was higher, averaging 81%. Coliform, fecal streptococci, and BOD removals were quite similar, averaging 68, 75, and 72%; however, COD removal averaged only 56%. It should be pointed out that most individual experiments were conducted for at least 120 h and in some cases for as long as 200 h (data not shown). Table 3 presents the results of a single typical experiment (experiment 4 with echovirus 12, Table 1).

The data in Table 3 show relatively good consistency in the virus-removing capacity of the filters for as long as 200 h of operation. Apparently the constant growth, sloughing, and regrowth of the biological slimes in these filters provided ample adsorption sites for the efficient removal of large numbers of viruses. Our assumption that virus removal by trickling filters is an adsorption phenomenon is based on (i) data obtained in virus removals by the activated sludge (6), (ii) the basic similarity of the two processes, (iii) the recognized resistance of these viruses to physical and chemical agents, and (iv) the very poor efficiency of sewage zoomicrobes in feeding on enteroviruses. Experiments were, therefore, conducted in an attempt to disassociate the adsorbed virus from the trickling filter “slime”. Several experiments were carried out in which “slime” was removed.

| Sample          | PFU/0.5 ml | Reduction (%) |
|-----------------|------------|---------------|
| Initial virus feed | 528,000 | —             |
| 3-h effluent     | 525       | 98           |
| 20-h effluent    | 1,100     | 96           |
| 27-h effluent    | 1,000     | 96           |
| 31-h effluent    | 1,760     | 93           |
| 51-h effluent    | 1,150     | 96           |
| 72-h effluent    | 2,540     | 90           |
| 96-h effluent    | 1,180     | 95           |
| 120-h effluent   | 4,400     | 82           |
| New virus feed   | 460,000   | —            |
| 144-h effluent   | 2,375     | 89           |
| 165-h effluent   | 2,150     | 90           |
| 168-h effluent   | 1,780     | 92           |
| 192-h effluent   | 1,400     | 94           |
| 200-h effluent   | 1,800     | 92           |

* Echovirus 12 equivalent filtration rate = 8 MGD/acre. Virus feed rate X = 326 ml/day. Virus dilution factor -

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\text{sewage feed rate + virus feed rate} = \frac{\text{virus feed rate}}{7,142} = \frac{326}{7,142} = 0.045
\]

Percentage of reduction = \( \frac{1 - \text{virus feed PFU}}{\text{effluent PFU}} \times 100 \)

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\text{effluent PFU} = 1 - \frac{326}{7,142} = 0.955
\]

**Table 2. Performance characteristics of rotary-tube trickling filters at high filtration rates**

| Virus            | Expt | Equivalent* filtration rate (MGD/acre) | Virus reduction (%) | Coliform reduction (%) | Fecal streptococcus reduction (%) | BOD reduction (%) | COD reduction (%) |
|------------------|------|----------------------------------------|---------------------|------------------------|----------------------------------|------------------|------------------|
| Poliovirus 1     | 1    | 25.0                                   | 58                  | 76                     | 74                               | 71               | 55               |
|                  | 2    | 23.5                                   | 75                  | 88                     | 83                               | 64               | 56               |
|                  | 3    | 23.8                                   | 43                  | 62                     | 68                               | 80               | 61               |
|                  | X    | 24.1                                   | 59                  | 75                     | 75                               | 72               | 57               |
| Echovirus 12     | 1    | 24.8                                   | 58                  | 88                     | 75                               | 66               | 53               |
|                  | 2    | 23.8                                   | 59                  | 56                     | 84                               | 76               | 46               |
|                  | 3    | 26.0                                   | 73                  | 44                     | 62                               | 75               | 58               |
|                  | X    | 24.9                                   | 63                  | 63                     | 74                               | 72               | 52               |
| Coxsackievirus A9| 1    | 20.8                                   | 74                  | 44                     | 62                               | 73               | 54               |
|                  | 2    | 19.5                                   | 96                  | 88                     | 88                               | 64               | 62               |
|                  | 3    | 20.8                                   | 72                  | 67                     | 75                               | 73               | 64               |
|                  | X    | 20.4                                   | 81                  | 66                     | 75                               | 70               | 60               |

* Six-foot deep bed of 2-inch stone.
from the rotary tubes after they had been fed sewage and virus for varying periods of time. The slime-virus complex was dispersed using a blender after the pH had been adjusted to between 8.0 and 8.3 with 1 N NaOH. Samples of the blended mixture were assayed for virus immediately after blending and after settling for about 1 h. In none of these experiments was any significant amount of virus recovered; this indicates that, as with activated sludge, the slime-virus complex is very stable or that the virus is somehow inactivated by adsorption to the “slime”.

Analyses of variance were applied to the data in Tables 1 and 2, and the results are presented in Table 4. The values of F (Table 4) for the different sources of variation indicate that the average percentages of reduction of viruses, coliforms, streptococci, BOD, and COD in the experiments carried out with each of the three enteroviruses at the medium filtration rate do not differ significantly, even at the 10% probability level. At filtration rates of 23 MGD/acre, there was more dispersion around the mean values (“within virus boxes”) because of the greater expected variation in virus quantitation, but the F test indicates that there was no additional detectable variation because of differences in the average percentage of recovery of different viruses or other contaminants at the 10% probability level.

To determine if there was a significant difference in virus-removing efficiency between the two filtration rates, the virus data in Tables 1 and 2 were subjected to the Student’s t test; the value of t was found to be 3.459. With n = 19, the P value is 0.005, which is far outside the 0.05 critical value. Thus, the trickling filter process operating at the medium filtration rate is significantly more efficient in virus removals than is the high filtration rate; in fact the same relative efficiencies were observed with coliform, streptococci, BOD, and COD removals.

**DISCUSSION**

The data presented in this paper indicate that the trickling filter process, as conducted in the laboratory with a bench-scale rotary-tube trickling filter, will partially remove at least three types of enterovirus from sewage, even in the absence of recirculation. The percentage of virus removed was less than that observed with a bench-model activated sludge unit (6). The activated sludge unit removed about 92% of poliovirus 1 at volatile solids loads of 600 to 4,000 mg/liter and 98% of coxsackievirus A9 at volatile solids loads of 600 to 1,500 mg/liter. In contrast, the rotary-tube trickling filters, operated at medium filtration rates (8.5 to 11.5 MGD/acre) removed about 85% of poliovirus 1 and about 94% of coxsackievirus A9. At high filtration rates (19.5 to 26.0 MGD/acre), 59% of the poliovirus 1 and 81% of the coxsackievirus A9 were removed. Echovirus 12 removal was studied only in the trickling filter system and the percentage of removals was almost identical.

**Table 4. Analysis of variance of percentage of reduction data in Tables 1 and 2**

| Source of variation | Sum of squares | Degrees of freedom | Estimated variance | F ratio     |
|---------------------|----------------|--------------------|--------------------|-------------|
| Between virus, coliform, streptococci, BOD, and COD reduction means | 435 | 4 | 109.0 | Nonsignificant at 10% probability |
| Interaction (residual) | 320 | 8 | 51.0 | Nonsignificant at 10% probability |
| Within boxes | 1,331 | 45 | 29.6 |               |
| Between virus, coliform, streptococci, BOD, and COD reduction means | 1,682 | 4 | 415.5 | Nonsignificant at 10% probability |
| Interaction | 935 | 8 | 52.3 | Nonsignificant at 10% probability |
| Within boxes | 4,439 | 30 | 165.2 |               |

* ± Standard deviation.
to poliovirus 1 removals. Student’s t distribution test was applied to the experimental data to determine if the activated sludge process was more efficient in removing viruses than the medium-rate trickling filter process. The value of P was found to be less than 0.001 for poliovirus 1 and 0.005 for coxsackievirus A9. Thus, the activated sludge process, as studied under laboratory conditions, is more efficient than the trickling filter process at medium filtration rates. These findings agree with those observed in field studies (9, 10, 13). It is possible that the trickling filter process with recirculation would achieve more consistent and greater virus removals than those observed with a single pass. The quantitative data obtained in our experiments do indicate that filtration rate is a very important factor affecting efficiency of the treatment process. At medium filtration rates, virus removal approached that of the activated sludge process; high-rate filtration significantly reduced the virus removal. It should be noted that the virus loads applied to these trickling filters (and in our previous studies with activated sludge) were several orders of magnitude greater than the numbers of viruses expected in raw urban sewage. These high virus loads were necessary to permit good quantitative assays of the treated effluent.

Coliform and fecal streptococci removals at medium filtration rates were about the same as those observed with the bench-model activated sludge unit; but as with the viruses, removal dropped significantly at the high-rate filtration, as did BOD and COD reductions.

Interestingly, in our analysis of variance in Table 4, the variations in removal “within boxes” were generally somewhat greater at the high filtration rates than at the medium filtration rates. At medium rates, the percentage of reduction in all categories except poliovirus 1 and echovirus 12 are well within expectation. The greater dispersion in percentage of removal of poliovirus 1 and echovirus 12 is not surprising since Berg et al. (3) demonstrated that, even under well-defined conditions, the percentage error (standard deviation divided by the mean and multiplied x100) generally fluctuated about the mean by 17%. Although the poliovirus 1 and echovirus 12 boxes showed more variation than the other boxes, this was because of the amazingly small values for standard deviations in their means. Actually, the dispersions in the poliovirus 1 and echovirus 12 boxes lie within the expected experimental error. We believe, therefore, that it can be generalized that the coliform and fecal streptococci reductions in this type of sewage treatment process can be used as an index of entero virus reduction. Disinfection, however, must be used to ensure a final effluent that is virus free.

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