



Project Summary

Virus Inactivation in Wastewater Effluents by Chlorine, Ozone, and Ultraviolet Light

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Virus levels before and after disinfection were studied in four full-scale wastewater treatment plants: two used chlorine as the disinfectant, one used ozone, and one used ultraviolet light. A second ozone study was conducted on a pilot scale at one of the treatment plants.

Results for the viral content in wastewater effluents indicated no consistent correlation between virus concentrations and any of the "traditionally" measured indicators of pollution. No seasonal variation of virus concentration was detected in this four-season study. A diurnal variation, however, was noted in one of the plants during two separate samplings: one in December and one in July. Maximum virus concentrations occurred in this effluent between 2:00 and 4:00 a.m. for each of these two sampling periods.

Ten different virus types were isolated. Poliovirus 1 was the predominant virus type found in both the treated and treated-plus disinfected effluents.

Experiments with pure strains and known quantities of attenuated viruses to determine recovery efficiencies indicated that variability in viral seed recovery data, although fairly small in a controlled laboratory environment, is more marked in a field situation.

This Project Summary was developed by EPA's Municipal Environmen-

tal Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Wastewater Treatment Plants

Four treatment plants were studied during this program: one each in Cincinnati (Ohio), Estes Park (Colorado), Marlborough (Massachusetts), and Waldwick (New Jersey).

The Muddy Creek Works (Cincinnati), studied in December 1976,¹ is a secondary treatment facility using a conventional activated sludge system followed by disinfection with chlorine. Effluent samples were collected both before and after applying the disinfectant. Virus content as well as the "traditional" water quality parameters were measured. The chlorine dose produced average total chlorine residual of 1.2 mg/L, after a contact time of 50 min. In July 1977,² ozone was applied as an alternative disinfectant for this same effluent. The effluent was transported by tank truck to the Robert A. Taft Laboratory (Cincinnati), the location of EPA's pilot ozone test facility. The packed-column ozone contactor used an applied ozone dose of 8 mg/L with a contact time of 30 sec.

The Estes Park Upper Thompson Sanitation District Treatment Plant (UTSD), sampled during the spring of 1977, consists of a conventional activated sludge plant followed by second stage attached growth nitrification, tri-media filtration, and disinfection with ozone. Ozone was produced from air using a corona discharge generator. The contactor was a baffled, closed, rectangular tank in which effluent flowed both concurrent and countercurrent with ozone, which was injected through porous stone diffusers placed at the bottom of the tank. The contact time at average flow was 37 min. Changes in operating conditions permitted a study to be completed that indicates dosage rates of approximately 5 mg/L are required to ensure virus die-off.

The Marlborough Easterly Wastewater Plant has a two-stage activated sludge system for nitrification followed by disinfection with chlorine. The effluents collected and studied from this plant in October 1977 had an average total chlorine residual of 1.3 mg/L after a contact time of 50 min.

The Northwest Bergen County Treatment Plant (Waldwick), sampled during April and May 1978, is a conventional and/or step aeration activated sludge system followed by disinfection with ultraviolet radiation. Although this plant has a design flow of 32,100 m³/d (8.5 mgd), during these studies the flow was only 19,000 m³/d (5 mgd). Clarified secondary effluent flowed through an ultraviolet disinfection unit manufactured by Pure Water Systems, Inc. This prototype unit consisted of a stainless steel rectangular compartment housing 400 ultraviolet lamps that were protected from the effluent by 23-mm (0.9-in. O.D.) quartz jackets spaced 12.7 mm (0.5 in.) apart. Contact time was approximately 3.6 sec.

Experimental Procedures

An Aquella* virus concentrator (Carborundum Company) mounted inside a mobile field laboratory concentrated waterborne viruses from large samples on a continuous basis. To ease handling and shipping problems, samples of approximately 380 L (100 gal) of effluent were concentrated to as small a volume of liquid as practicable for analysis. Sodium thiosulfate, dilute HCl, and AlCl₃ were added to the effluent sample,

and the solution passed through virus-adsorbing filters. These filters were removed and placed into 1 to 3 L of glycine solutions; the solution was further concentrated and stored in fetal calf serum at dry ice temperatures before shipment to the virus assay laboratory.

Analysis of samples for natural virus was carried out on two types of cell cultures: a continuous cell line (Buffalo Green Monkey, BGM) maintained at the University of New Hampshire virus laboratory and a primary cell line (African Green Monkey Kidney, PMK), which was prepared fresh for each sample. The virus assays were made by plaquing methods that permitted separation and enumeration of viruses present in the samples. Virus isolates were identified by serum neutralization tests using eight Lim-Benyesh-Melnick (LBM) anti-serum pools.

Results

Virus isolation rates were determined for each of the samples by equally weighting each sample that contained one or more virions. The data from each sample were then tabulated to form the reported average. Based on this weighting, 38% of all samplings, before disinfection, contained one or more virions. The actual virus isolation rates for the effluent before disinfection were: UTSD Treatment Plant, 69%; Muddy Creek Works, 75%; Taft Pilot Plant, 57%; Marlborough Easterly Plant, 38%; Northwest Bergen County Plant, 56%.

There were no major differences in seasonal isolation rates, particularly for the two sampling periods completed in July and December at the Muddy Creek Works. The latter two rates for non-disinfected effluent were 75% and 60%, respectively.

Significantly lower isolation rates were encountered after disinfection. Reductions occurred for each of the three disinfectants (ultraviolet, chlorine, and ozone) studied. The actual virus isolation rates for the effluent after disinfection were: UTSD Treatment Plant (all sample days), 50%; UTSD (eliminating 4 days of deliberately decreased ozone dosage), 33%; Muddy Creek Works, 38%; Taft Pilot Plant, 7%; Marlborough Easterly Plant, 0%; Northwest Bergen County Plant, 13%.

Virus isolation rates were reduced 75% when ultraviolet light was used as a disinfectant; 50% when ozone was used at UTSD; 88% when ozone was used for Muddy Creek at Taft; and 50%

and 100% (none detected) for the two chlorine samplings. Similar results were obtained by analyzing the same data using virus titer rather than isolation rates.

The relative distribution of viruses by classification, based on the total number of viruses in all non-disinfected effluents, was measured. The polio and coxsackievirus types were isolated and individually identified. All three types of polioviruses (1, 2, and 3) were shown to be present in the effluents. These viruses were not assayed to determine their virulence. Coxsackie A9, B1, B2, B3, B4, B5, and B6 were also isolated and identified during this program. Except for the coxsackie B5 virus, all virus types were found in the treated as well as the treated plus disinfected effluent.

Virus levels in treated effluent varied diurnally in one of the plants—the Muddy Creek Works. Virus concentrations were greater (as high as 40 viruses per 380 L (100 gal)) between 2:00 and 4:00 a.m. in both December and July. An increase of almost an order of magnitude was found for virus titers between 2:00 and 4:00 a.m. compared with measurements during the remainder of the day. This increase remains unexplained.

The diurnal variations demonstrated at the Muddy Creek Treatment Plant provided a unique opportunity for comparisons with the traditionally measured water quality indicators. Linear regression analyses performed on the July data determined indicators that might correlate with virus levels. Total coliform, fecal coliform, chemical oxygen demand, and total organic carbon had a positive correlation coefficient. No correlation was evident for total suspended solids and turbidity.

A similar analysis was performed of effluent data collected in December. In this instance, no correlation was found with total coliforms and fecal coliforms whereas a positive correlation with total suspended solids and turbidity occurred. Both COD and TOC exhibited a positive correlation with virus titers in December as well as July.

Conclusions

In general, the present study provides a framework for analyzing the effectiveness of the various disinfectants to inactivate viruses. Questions in many areas, however, remain unanswered. The amount of data required to provide a statistically accurate profile on the relative disinfection abilities of chlorine,

*Mention of trade names or commercial products does not constitute endorsement for use by the U.S. Environmental Protection Agency.

zone, and ultraviolet radiation are attainable only if further sampling studies are undertaken as part of a coordinated research program.

Because virus levels encountered in each of the tested effluents *before* disinfection were extremely low, detection of significant reductions *after* disinfection was very difficult. In all cases, reductions were noted, but the parameter used to measure these reductions was virus isolation rate (i.e., the number of samples positive for virus divided by the total number of samples tested per treatment plant), not virus titer. Thus, the trend was there, but only qualitatively.

The low levels of indigenous viruses in the undisinfected effluents were not anticipated. There was also a further complication. Seeding experiments with attenuated poliovirus strains at each of the treatment plants indicated that the method of concentrating large volumes of effluent (380 L) down to 1 to 2 L was quite inefficient (approximately 10%) and exceedingly variable. This made quantitative analysis and interpretation even more difficult.

There is still a need to determine how well disinfectants inactivate indigenous viruses under actual operating conditions. This study, however, clearly demonstrated that virus recovery techniques need considerable improvement. Field studies should be undertaken only after a viable measurement technique exists.

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The complete report, entitled "Virus Inactivation in Wastewater Effluents by Chlorine, Ozone, and Ultraviolet Light," (Order No. PB 81-208 183; Cost: \$9.50, subject to change) will be available only from:

*National Technical Information Service
5285 Port Royal Road
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