Recovery of *Escherichia coli* from Chlorinated Secondary Sewage

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*Escherichia coli* ATCC 27622 injured during chlorination of secondary sewage failed to produce colonies on membrane filters incubated on m-FC medium or to grow and produce gas in lactose broth. Thus, standard methods for the enumeration of total and fecal coliforms in water and wastes should not be applied to chlorinated effluents.

There is a trend towards the use of bacteriological assays of the efficacy of chlorination of secondary sewage effluents. If effluent standards are to place limitations upon discharges of indicator bacteria, dependable standardized methods must be employed for their enumeration. McKee et al. (3) demonstrated significantly poorer recoveries of coliforms from chlorinated settled sewage by membrane filter techniques than by most probable numbers (MPNs). Lin (2) also demonstrated higher MPNs of total coliforms in chlorinated secondary effluents, but showed that the discrepancy could be eliminated by incubating membranes on LES ENDO medium prior to transfer to m-ENDO medium. When Lin compared recoveries of fecal coliforms from chlorinated secondary effluents, the MPN procedure again was superior to the membrane filter technique.

An explanation for the observations of these investigators is suggested by the work of Maxcy (4) and Scheusner et al. (5), who showed that *Escherichia coli* injured during chlorination failed to form colonies on a selective medium. The present studies were undertaken to examine injury to *E. coli* during chlorination of secondary sewage and to evaluate the efficacy of standard procedures for the enumeration of fecal coliforms in chlorinated effluents.

Secondary effluent was obtained from the sewage treatment plant on the University of Florida campus prior to chlorination and was sterilized in the autoclave for 20 min at 15 lb/in². Cells of *E. coli* ATCC 27622 were harvested after 24 h of growth at 37 °C on the surface of nutrient agar plates and were added to 500 ml of sterile sewage in a sterile 750-ml beaker to yield a population of approximately 50,000/ml. Sewage was maintained at room temperature, which remained between 21 and 22 °C during all experiments. Chlorine was added as NaOCl at dosages varying between 2 and 3 mg/liter. Dosages were determined on the basis of chlorine demand tests, so that a total residual of 0.3 to 0.5 mg/liter remained after 30 min of contact. Samples (10 ml) were removed after 1, 5, 10, 20, and 30 min of contact and were placed immediately in 10 ml of sterile sodium thiosulfate (50 mg/liter) and diluted appropriately. Spread plates were prepared in triplicate on Trypticase soy agar (BBL). MPNs were determined on 5-tube serial dilutions in lactose broth (1) and in EC broth incubated at 44.5 °C. Membrane filters were incubated on m-FC medium according to standard methods (1).

Results obtained in typical experiments are presented in Fig. 1 and Table 1. Decreases in viable counts obtained by all methods tested occurred exponentially, although a lag always preceded the exponential phase measured on Trypticase soy agar. The decrease in viable count determined by the membrane filter technique took place at a higher and more variable rate than that obtained by other techniques, as it has been observed to do in aqueous suspension (2a). In general, curves intersected at zero time, indicating that recovery of unstressed cells occurred equally well by all methods. The absence of a lag, and higher rates of decrease in viable counts by the two MPN procedures and membrane filter technique, suggest that injury prevented recovery of a substantial portion of the viable population when the latter techniques were employed.

Thus, counts of fecal coliforms in chlorinated secondary effluents measured by currently em-
counts increases with length of exposure to chlorine. If counts of indicator bacteria in chlorinated secondary effluents are required by regulatory agencies, more reliable methods of enumeration must be developed.

**LITERATURE CITED**

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