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Evaluation of Methods for Detecting Coliforms and Fecal Streptococci in Chlorinated Sewage Effluents

by S. D. LIN

ILLINOIS STATE WATER SURVEY

URBANA

1974

REPORT OF INVESTIGATION 78

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ABSTRACT: Total coliform (TC), fecal coliform (FC), and fecal streptococcus (FS) recoveries in chlorinated secondary sewage effluents were investigated by the membrane filter (MF) and multiple-tube (most probable number, MPN) methods. The LES two-step MF method was found to be comparable to the MPN procedure for determining TC. The TC detection was 1.5 times greater by the LES two-step technique than that obtained by the M-Endo one-step MF procedure. Fecal coliform recovery by the M-FC MF procedure was lower than the recovery obtained by the MPN method. Azide-dextrose broth, brain-heart infusion broth, and peptone yeast-extract casitone used separately with the M-Enterococcus agar MF_2 (2-day incubation) procedure were not satisfactory for the recovery of FS. The M-Enterococcus agar procedure with bile broth enrichment (MF_2) or prolonged incubation for 3 days (MF_3) significantly increased FS recovery and both were comparable to the MPN method. The results cited should be useful in assessing the efficiency of disinfection practices for waste treatment plants employing effluent chlorination.

REFERENCE: Lin, S. D. Evaluation of Methods for Detecting Coliforms and Fecal Streptococci in Chlorinated Sewage Effluents. Illinois State Water Survey, Urbana, Report of Investigation 78, 1974.

INDEXING TERMS: bacteria, chlorination, enrichment procedure, environmental engineering, fecal coliforms, fecal streptococci, MF and MPN methods, prolonged incubation, sanitary engineering, secondary sewage effluents, total coliforms, water pollution.

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URBANA
1974

Printed by authority of the State of Illinois-Ch. 127, IRS, Par. 58.29
(10-74-1500)

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Evaluation of Methods for Detecting Coliforms and Fecal Streptococci in Chlorinated Sewage Effluents

by S. D. Lin

SUMMARY AND CONCLUSIONS

Two series of laboratory assays were performed to determine whether or not the results with the standard membrane filter (MF) procedure for total coliform, fecal coliform, and fecal streptococcus detections on chlorinated secondary sewage effluents were comparable to those obtained by the multiple-tube (MPN) method.. If not found to be the case, efforts were made to improve bacteria recoveries by various modifications of the MF method.

Grab samples of secondary effluents were collected from three Illinois treatment plants. They were chlorinated with as much as 6 mg/1 of chlorine, stirred, and dechlorinated by sodium thiosulfate. After varying periods of contact, the samples were assayed for bacteria. On the basis of the results derived from this work, the following conclusions were drawn.

For chlorinated secondary sewage effluents, the recoveries of TC, FC, and FS by the standard MF (one-step nonenrichment) method are significantly less than those obtained by the standard MPN procedure.

The use of the LES two-step MF method is comparable to the completed MPN procedures for total coliform detection. Total coliform recovery by the LES two-step MF technique is approximately 1.5 times that obtained using the M-Endo, one-step MF procedure. From 273 filters with the use of the LES two-step MF procedure and 1110 sheen colonies, 89.6 percent were verified as coliform organisms.

Estimates of MPN FC densities may be derived from the MF procedure by use of a mathematical relationship similar to log MPN = 1.06 log MF — 0.01. For FC verification, 87.7 percent of 616 blue colonies were verified.

Azide-dextrose broth, brain-heart infusion broth, and peptone yeast-extract casitone used separately for enrichment purposes with the M-Enterococcus agar MF_2 procedure did not satisfactorily increase the sensitivity of the procedure for FS assays. Enrichment with bile broth medium of the M-Enterococcus agar MF_2 procedure significantly increases the FS recovery to the extent that the procedure is comparable to the multiple-tube method.

The recovery of FS by the membrane filter technique with M-Enterococcus agar increased significandy after 3 days incubation (MF_3) compared with 2 days incubation (MF,), and the MF_3 procedure is comparable to the multiple-tube method for FS detection. The membrane filter technique preferred for FS assays is the MF_2 procedure using M-Enterococcus agar with bile broth enrichment. All of 688 colonies for 2-day incubation on filters were verified as fecal streptococci.

INTRODUCTION

The year-round disinfection of wastewater treatment plant effluents has become mandatory in Illinois and in several other states. The most common method of disinfection at treatment plants is chlorination. Its effectiveness has generally been measured by residual chlorine. The Illinois Pollution Control Board[1] requires a limitation on fecal coliform (FC) densities independent of residual chlorine thus requiring determinations for FC densities in chlorinated effluents. The Board's rules stipulate that fecal coliform densities in a waste effluent shall not exceed 400 per 100 milliliters (ml).

Total coliforms (TC) have been used for measuring the disinfection efficiencies of water and wastewater treatment units. The TC index is still valid and reliable for the water industry. In European countries fecal streptococci (FS) are commonly looked for in the sanitary analysis of water supplies.[2] In the United States, they are used currently in conjunction with FC for determining the sanitary quality of water. Although FS determinations are not required by most regulatory agencies, the usefulness of the procedure should not be overlooked.

The requirement for bacteria enumeration in treated effluents necessitates the development of adequate and economical procedures for determining bacteria densities in chlorinated effluents. The series of investigations described in this report were undertaken with that objective in mind.

Literature Review

Indicator Organisms. The purpose of the routine bacteriological examination of water samples is usually to estimate the hazard due to fecal pollution and the probability

of the presence of pathogenic organisms. The isolation of pathogens from water and sewage is expensive and laborious. It is not a routine practice. Normally occurring bacteria in the intestines of warm-blooded animals have been used as indicators of fecal pollution. Total coliforms, fecal coliforms, and fecal streptococci have all been used as pollution indicators at various times.[3,4] Other bacterial indicators have been proposed including *Closteridium, Pseudomonas,* and *Aerobacter,* but their value has been considered questionable or irrelevant.[5]

Correlations between coliforms and pathogenic bacteria have been cited frequently, i.e., coliforms vs *Salmonella.*[6,7,8,9] Less known is the relationship, if any exists, between coliforms and viruses. A coliform index is not a reliable index for viruses.[10,11] There is little evidence that enteroviral or other microbial diseases are transmitted frequently by the drinking water route in the absence of coliforms.[5]

Until more definitive studies are completed on the relationship of pathogens and indicator organisms, the use of TC for water supplies and FC and FS for sewage and stream quality, as indicators of enteric pollution, is valid.

Total Coliforms. Total coliform densities have been used to measure the occurrence and degree of fecal pollution in streams for over 60 years. As defined in *Standard Methods,*[4] "the coliform group comprises all of the aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hr at 35°C."

The TC group has been adopted as an indicator of fecal pollution suggestive of a hazard to health because these bacteria are associated with the gut of warm-blooded animals. Thus, the absence of TC is generally evidence of a bacteriologically safe water.

The TC group can be subgrouped as fecal and nonfecal coliforms. The fecal coliform subgroup is derived from feces of human and other warm-blooded animals such as cows, sheep, poultry, etc. The other (nonfecal) subgroup is frequently found on vegetation and in the soil; some are plant pathogens. Organisms of the nonfecal subgroup tend to survive longer in water than do the fecal subgroup. The nonfecal coliforms also tend to be somewhat more resistant to chlorination than the FC group or the commonly occurring intestinal bacterial pathogens.[12] The aftergrowth of TC organisms is generally associated with the *Aerobacter aerogenes* portion of the nonfecal subgroup.[13,14] Therefore the sanitary significances of these two subgroups are different. The presence of FC organisms indicates recent, and possibly hazardous, fecal pollution. The presence of nonfecal coliforms suggests less recent pollution or reveals defects in water treatment or distribution systems.[3]

Fecal Coliforms. If the hypothesis that the coliform bacteria of fecal origin represent greater danger to health than those native to other environments is accepted, the separation of the fecal and nonfecal groups is necessary. Enumerating methods for FC by the elevated temperature tests have been developed by Geldreich et al. for the MPN procedure[15] and for the MF technique.[16] The MF technique with the FC medium is an acceptable procedure listed in the 13 th edition of *Standard Methods.*[4] It detects not only *E. coli* but other coliform types that are derived from warm-blooded animal feces.

The most common fecal coliform species is *Escherichia coli.* The FC organisms generally do not multiply outside the intestines of warm-blooded animals, except in certain high-carbohydrate wastewaters such as that from sugar beet refineries.[5] Populations and types vary from host species to host species, and even according to the individual.[17]

In domestic sewage, the FC density may constitute 30 to 40 percent of the TC density. In aged sewage and in polluted waters, the FC fraction tends to decrease progressively with elapsed time. In heavily polluted surface waters, the FC component usually falls between 10 and 35 percent of the TC count.[3] In natural waters, relatively free from recent pollution by enteric wastes, the FC count is unlikely to exceed 10 percent of the TC count. There are, however, too many variables relating to enteric pollution, runoff water, and natural water quality, to permit a sweeping generalization on the numerical relationships between FC and TC.

Fecal Streptococci. The fecal streptococci group, as stated in *Standard Methods,*[4] is restricted to the following species, or their varieties: *S. faecalis, S. faecalis var. liquefaciens, S. faecalis var. zymogenes, S. durans, S. faecium, S. bovis,* and *S. equinus.* The 'enterococcus' refers to a more restrictive group, including all the above species except *S. bovis* and *S. equinus.* The terms 'fecal streptococcus' and 'Lancefield's Group D streptococcus' are considered synonymous.

In the present state of knowledge, a precise definition of fecal streptococci is not possible. The United Kingdom Ministry of Health defines these organisms as "gram-positive cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45°C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria."[5] However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water." Some workers consider that growth at 45 C and multiplication in 40 percent bile broth are the most significant indications of fecal origin of streptococci.

Fecal streptococci are nonpathogenic organisms. Nevertheless their common occurrence in the intestines of man and other warm-blooded animals makes them a useful group as an indicator of fecal contamination. They also have been considered indicators of fecal pollution for nearly 60

years.[18,19] Their poor acceptance as a pollution indicator is due to low recovery rates, the multiplicity of detection procedures, poor agreement between various enumeration methods, and the lack of detailed and systematic studies of the sources, survival, and interpretation of FS in various kinds of water. On the basis of more recent studies the FS will become an additional indicator particularly valuable for stream studies.

Bartley and Slanetz[20] reported that organisms producing large maroon colored colonies on membranes are usually the tellurite resistant 5. *faecalis* types of FS, and the small pink colonies are usually tellurite sensitive *S. bovis* types. The confirmation of these can be made by Gram stain and appropriate culture tests. At least 80 percent of FS from human origin is of the *S. faecalis* groups. The major groups found in the feces of most domestic animals, especially cows and sheep, are S. *bovis* and 5. *equinus.*[17,21] This diversity in grouping, dependent upon source, permits reasonable estimates of the sources (animal versus human) of fecal contamination in water. FS will not multiply in water but some species may survive in unfavorable conditions. Their die-off rate is uncertain at this time.[17]

Studies[22] have indicated the concentration of FS in feces to be of the same order of magnitude as that of conforms. The isolation and enumeration procedures satisfactory for routine laboratory tests are available. On the basis of these advantages, the Committee on Public Health Activities of the American Society of Civil Engineers[22] concluded that the use of FS is superior to coliform organisms as pollution indicators.

Geldreich et al.[23] first suggested the use of an FC to FS ratio as a more valuable informational tool for assessing pollution sources than the use solely of FC densities. In applying the ratio concept to a natural stream system, stream samples not more than 24 hours downstream of a pollution source must be used. Ratios greater than 4:1 indicate the pollution source is likely to be derived from domestic wastewaters, whereas ratios less than 0.7:1 suggest the bacteria are from sources other than human, i.e., livestock and poultry wastes.[14,24] With these considerations in mind, together with other suggested interpretations for intermediate values,[17] FS determinations can be an important tool for a stream study.

Although there has not been any definitive work reported on FC:FS ratios for chlorinated sewage effluents, some preliminary results[25] suggest that FS organisms might be more resistant to chlorination than FC types. On unchlorinated effluents the FC:FS ratio was found to average 11.2:1 for 18 samples; on chlorinated effluents the ratio averaged 0.72:1 for 63 samples.

Bacteria Enumeration. The basic methods for the assay of pollution indicators (TC, FC, and FS) in waters are outlined in *Standard Methods.*[4] These include the multiple-tube or most probable number (MPN) technique and the membrane filter (MF) procedure. *Standard Methods,* however, states that "Experience indicates that the MF procedure is applicable to the examination of saline waters but not chlorinated wastewaters." Because the MF technique is comparable to the MPN procedure and is less time consuming, it seems unfortunate that the MF technique cannot be used as a control procedure by the waste plant operator who uses chlorination.

McKee et al.[26] reported on the lack of correlation between MPN and MF techniques while assaying chlorinated settled wastewater for total coliforms. Because monochloramine is the predominant bactericidal agent in chlorinated wastes, they advanced the hypothesis that partial reversibility is responsible for the discrepancy between MPN and MF results; that is, the MF technique produces considerably fewer colonies than the number that develop by the MPN method. Presumably, when inactivated cells are deposited on a membrane with limited nutrient availability, the cells cannot rid themselves of monochloramine and therefore cannot grow. However, when inactivated cells are put in an aqueous medium rich in organic matter, such as lactose broth, the monochloramine may diffuse outwardly from the cells, permitting them to recover, grow, and produce gas.

In the McKee et al.[26,27] investigations, dehydrated scheduled nutrient (DSN) pads were used for the MF technique. They contained two elements with an upper leaf impregnated with an Endo-type inhibitory nutrient. The results obtained from the use of DSN pads with the MF technique were comparable to those obtained from the confirmed MPN procedures on raw settled wastewater. McCarthy et al.,[28] though working initially with water, were not satisfied with the one-step, M-Endo broth MF techniques. Their work suggested that enrichment plus an agar substrate was superior to the one-step technique on the basis of a higher degree of coliform recovery. Examinations of natural waters and wastewater demonstrated that these results were comparable to standard MPN data. From their work an agar-based medium (LES M-Endo agar) was developed. Its use with the MF technique is basically a two-step enrichment procedure.

The need has developed not only for determining total coliforms but also for enumerating fecal coliform densities. Geldreich et al.[16] recommended the use of an M-FC medium at incubation temperatures of 44.5 ±0.5 C as part of the MF technique for the direct count of fecal coliforms. It has been reported[29,30,31] that the determinations for fecal coliforms rather than total coliforms are a more realistic measurement of the public health significance of microbial discharges in wastewater plants. Illinois requirements specify maximum permissible limits for fecal coliform concentrations in treated effluents. This will require fecal coliform enumeration in chlorinated effluents.

Lattanzi and Mood [32] used the Winter and Sandholzer

method for the detection of enterococci. Later Litsky et al.[33] suggested the use of glucose azide broth as a presumptive medium and ethyl violet azide (EVA) broth as a confirmatory medium for enterococci detection with MPN procedures.

Slanetz and Bartley[34] proposed the use of M-Enterococcus agar for the isolation of FS by the MF method. Kenner et al.[35] introduced the KF streptococcus agar. Rose and Litsky[36] found they could increase the recovery of FS from river water by more than 2-fold when using peptone yeast-extract casitone (PYC) compared with M-Enterococcus agar. Recently Pavlova et al.[37] suggested that fluorescent antibody techniques may be useful for FS detection, in determining the presence and source of fecal pollution in water.

Objectives and Report Plan

During this study two separate investigations were performed. One dealt principally with TC and FC, the other with FS. The purposes of the study were:

1) To determine whether or not the MF technique for TC, FC, and FS detections in chlorinated secondary effluents is comparable to the MPN method.
2) To determine whether or not the LES two-step enrichment MF technique for TC detection, in chlorinated secondary effluents, is comparable to recommended MPN methodology.
3) To develop improvements in the MF method for the detection of FS in chlorinated secondary sewage effluents.

This report describes the procedures used. It also includes the results obtained and a discussion for each of the bacterial groups examined, i.e., TC, FC, and FS. The two-step enrichment MF method is described in appendix A. Included in appendices B, C, and D are tabulations of observed data for TC, FC, and FS, respectively.

Acknowledgments

The study was conducted under the general supervision of Ralph L. Evans, Head of the Water Quality Section, and Dr. William C. Ackermann, Chief, Illinois State Water Survey. A special expression of gratitude is extended to Dorothea Seiz, Microbiologist, Illinois Department of Public Health, Springfield, for her valuable advice. Many Water Survey personnel assisted in the study. Davis B. Beuscher, Jack W. Williams, Pamella A. Martin, Donald H. Schnepper,. and Larry G. Epley assisted in laboratory and sampling during this study. Mrs. J. Loreena Ivens, Technical Editor, edited the final report; Mrs. Suzi O'Connor prepared the camera-ready copy; Miss Katherine Shemas, Clerk Typist, typed the original manuscript; and John Brother, Jr., Chief Draftsman, prepared the illustrations.

MATERIALS AND METHODS

Grab samples of final settling tank effluents from three wastewater treatment plants serving the cities of Peoria, Morton, and Washington in Illinois were used in the study. At least five effluent samples from each plant were examined. The Peoria plant employs the high-rate activated sludge process treating a combination of domestic and industrial wastewaters. Contact stabilization comparable to the standard-rate activated sludge process is used at Morton. This plant treats principally domestic wastewater. Washington is served by a standard-rate trickling filter plant, treating domestic wastewater also.

One-liter portions of each effluent were dosed with calcium hypochlorite (HTH, 70 percent available chlorine) up through 6 mg/1 of chlorine. The samples were stirred gently but intermittently, and after varying periods of contact (up to 30 minutes) they were dechlorinated with an excess of sodium thiosulfate. The dechlorinated samples were assayed immediately for bacterial densities by parallel MPN and MF methods.

The MPN procedures were performed by using a series of four decimal dilutions per sample, with five tubes for each dilution. Lauryl tryptose (LT) broth was used for the presumptive tests in TC and FC determinations. The TC test was confirmed with the use of brilliant green bile (BGB) medium, and was completed with Gram stain. For FC confirmation, an EC medium at 44.5 ±0.5 C (water bath) was used. In the MPN procedure for FS tests, azide-dextrose (AD) broth was used for the presumptive test, while ethyl violet azide broth was used for confirmation.

In the MF procedures for TC, FC, and FS, three duplications for each sample were filtered through an 0.45M membrane filter for each bacteria test. For TC tests, the two-step enrichment of LES M-Endo agar[28] was followed. Occasionally, parallel tests with the standard one-step M-Endo procedure were performed. For TC verification purposes, representative colonies (3 to 6 sheen colonies per filter) were subcultured through LT broth into BGB broth.[38] Production of gas on BGB broth was deemed verification.

When using MF procedures for FC detections, the recommendations of Geldreich et al.[16] were followed. Several colonies (3 to 6 blue colonies per filter) placed on a filter and incubated onto the M-FC medium were verified by in-

oculating in phenol red lactose broth for a 24- to 48-hour period at 35°C and noting gas production. All positive tubes were confirmed at 44.5 C in EC broth.

In the determination of FS densities by the MF technique, the standard one-step M-Enterococcus agar[4] was used. According to Seiz[39] M-Enterococcus agar is superior to KF streptococcus agar, for sewage effluents because some of the nonstreptococci species in sewage samples grow red and pink colonies on KF streptococcus agar. The FS counts on the membrane filters were generally made after 2, 3, 4, and 7 days incubation. Parallel tests with the two-step enrichment (appendix A) were also performed. The enrichment media used include AD broth, brain-heart infusion (BHI) broth, bile broth medium (prepared by adding 40 ml sterile 10 percent oxgall solution to 60 ml sterile BHI broth), and PYC broth. The period of the pre-enrichment was 2 to 3 hours. For the purpose of FS verification, red and pink colonies (3 to 6 colonies per filter) were fished at random from the membrane filter and inoculated onto a brain-heart infusion agar (BHIA) slant, followed by a catalase test. If the catalase test was negative, then the growth on the BHIA slant was subcultured into both a BHI broth and a bile broth medium for confirmation.

With slight variation all bacterial assay procedures followed *Standard Methods.*[4] Generally, all the media used were freshly prepared, and none was more than 4 days old.

RESULTS AND DISCUSSION

Total Coliforms

Multiple-Tube versus Membrane Filter. Consistent with *Standard Methods*[4] recommendations that a comparison be made between MPN and MF techniques before using the MF procedure, a series of bacterial assays on unchlorinated samples from a variety of sources was performed. This evaluation included enumeration for total coliforms as well as fecal coliforms. Table 1 summarizes the results. Results of the paired data t-test technique in testing the hypothesis (H_0) that the mean of the first population is equal to the mean of the second (tests 1 and 3 of table 9) did not indicate significant differences in TC and FC recoveries determined by the MPN and MF methods. The comparisons for the purposes of this study, therefore, were considered acceptable.

Table 1. Comparison of the MPN and the MF Coliform Densities of Unchlorinated Waters (Densities per 100 ml)

Source	Total coliforms		Fecal coliforms	
	Completed MPN	*LES-MF*	*MPN*	*MF*
Illinois River	1,700	1,400	230	640
	1,300	2,000	490	330
	790	1,200	170	270
			79	160
Spoon River	2,400	2,700	490	330
	3,300	3,100	170	270
	460	1,200	140	250
	1,300	2,600	790	790
Activated sludge process effluent	92,000,000	80,000,000	35,000,000	19,000,000
	13,000,000	12,000,000		
	7,900,000	7,400,000	4,900,000	1,000,000
	5,400,000	6,700,000	2,400,000	2,100,000
			460,000	400,000
	790,000	1,300,000	33,000	30,000
	350,000	670,000	79,000	52,000
	240,000	360,000	79,000	80,000
			49,000	59,000
			33,000	60,000
Trickling filter process effluent	3,500,000	4,000,000		
			490,000	600,000
			490,000	410,000
Tertiary pond effluent			790,000	600,000

M-Endo {one-step) versus LES M-Endo (two-step). Samples of chlorinated effluents from an activated sludge process were evaluated for TC densities by M-Endo one-step and LES M-Endo agar two-step MF procedures. McCarthy et al.[28] performed a similar assessment on unchlorinated water samples from rivers, lakes, and ponds leading to the development of the LES agar-based medium. The results obtained on chlorinated secondary effluents were comparable to those observed by McCarthy et al.[28] As shown in figure 1, the plotted data lie above the equality line, indicating that total coliform recovery by the LES two-step procedure was superior to the M-Endo one-step method. A better development of sheen colonies was also observed on the LES medium.

The experiences of McCarthy et al.[28] and McKee et al.[26] were similar with regard to total coliform recovery from unchlorinated wastewater samples. The McCarthy group found no advantage in using an enrichment phase when compared with a one-step agar method on unchlorinated wastes and polluted waters. They suggested that the recovery efficiency for total coliforms was a function of the number of coliforms in the sample. Therefore, in natural waters where smaller numbers of coliforms are likely to exist, an enrichment phase in the MF technique is required, whereas with polluted waters and unchlorinated wastewater, the enrichment two-step procedure can be omitted without significant

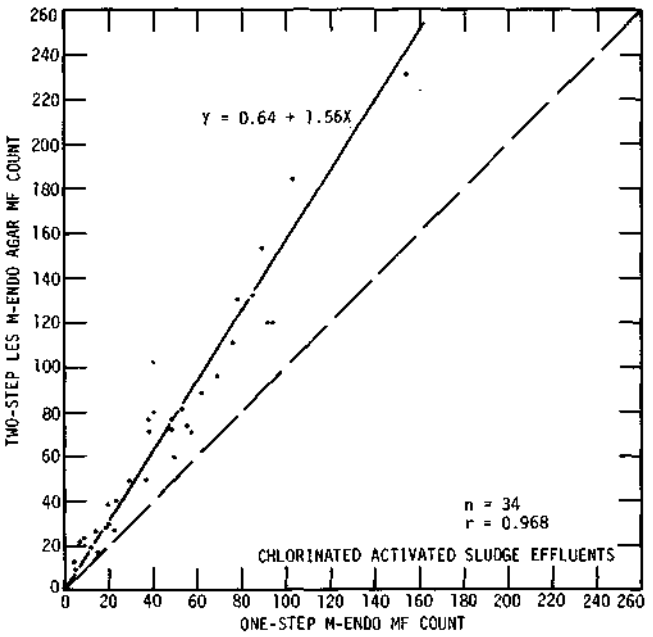

Figure 1. Comparison of TC counts made on M-Endo broth and on LES M-Endo agar

effect on coliform recovery. McKee and his colleagues experienced the lessening of coliform recovery on chlorinated settled wastewater similar to that described for water with a smaller number of coliforms. This suggests that equivalent conditions are encountered when temporarily inactivated colonies exist or a smaller number of colonies are present. In both cases, an enrichment phase would more than likely be required to attain satisfactory coliform recovery with the MF technique.

Although the number of colonies per filter as depicted in figure 1 exceeded the desirable range of 20 to 80 per filter, they were considered satisfactory for comparison purposes. The results correlated well (r = 0.968), and the relationship between the two procedures can be expressed as

$$TC_2 = 0.64 + 1.56\,TC_1 \qquad (1)$$

where TC_1 and TC_2 are, respectively, the total coliform colonies determined by the one-step and two-step MF techniques. The total coliform recovery on chlorinated effluents by the LES two-step procedure is about 1.5 times greater than that attained by the M-Endo one-step method.

LES (two-step) versus Multiple-Tube. The multiple-tube method is considered acceptable for assaying the total coliform densities in chlorinated wastewater effluents. A comparison of the total coliform data resulting from the LES two-step method, which was used in this study, with bacterial densities obtained from parallel multiple-tube observations was therefore pertinent. Methods described by Thomas[40] were used in figures 2 and 3 to depict the total coliform data for all chlorinated secondary effluents examined. Figure 2 represents observations of the LES two-step MF technique, and figure 3 represents observations of the multiple-tube procedure. The figures reflect simply the geometric distribution of the bacterial densities for all effluents as determined by the two techniques. Similar curves could have been presented for each type of effluent.

More important for comparative purposes is the summary included in table 2. For the MF technique, including all data, the geometric mean (M) was 29,000 total coliforms/100 ml; the geometric standard deviation (σ_g) was 12.14; and the arithmetic mean computed from geometric parameters[40] was 650,000/100 ml. Similarly, the MPN data reflected a geometric mean of 28,000 coliforms/100 ml, a geometric standard deviation of 13.55, and an arithmetic mean of 700,000/100 ml. All of the data, including that for each type of effluent summarized in table 2, suggest that the LES two-step MF method for chlorinated effluents is comparable in coliform recovery efficiency to the multiple-tube procedure.

Figure 4 is a graphical presentation for comparative purposes also. For the 71 samples examined, 32 of the MF results are higher and 34 of the MF results are lower than concurrent MPN results. Five observations were found to be identical. The ratios of MF:MPN varied from 0.44 to 5.03 with a median of 1.00. The mathematical relationship between the two procedures observed during this study was

$$\log MF = 0.26 + 0.94 \log MPN \qquad (2)$$

with a correlation coefficient of 0.99.

As shown in test 2 of table 9 the TC recovered by the MPN and LES M-Endo MF methods are not significantly different. It is concluded that the LES two-step MF technique was as good as the multiple-tube method for assaying total coliform densities in chlorinated secondary effluents. From the standpoint of time, convenience, freedom from bias, and equipment needs, the LES two-step technique would seem preferable to the multiple-tube technique for chlorinated effluents.

Table 2. Comparative Results for Total Coliform Data (Total coliforms per 100 ml)

Chlorinated effluent	Number of observations	Test*	Geometric mean	Geometric standard deviation	Arithmetic mean†
High rate activated sludge	21	MF	40,000	18.00	2,600,000
		MPN	36,000	20.43	2,900,000
Contact stabilization	24	MF	5,100	5.16	190,000
		MPN	4,600	5.20	150,000
Trickling filter	26	MF	110,000	6.31	6,000,000
		MPN	120,000	7.15	7,000,000
Total	71	MF	29,000	12.14	650,000
		MPN	28,000	13.55	700,000

* LES (two-step) MF and completed MPN

† $M = CM_g\sigma_g^{1.15 \log \sigma_g}$;
C = 1.0 for MF, c = 0.851 for 5-tube MPN

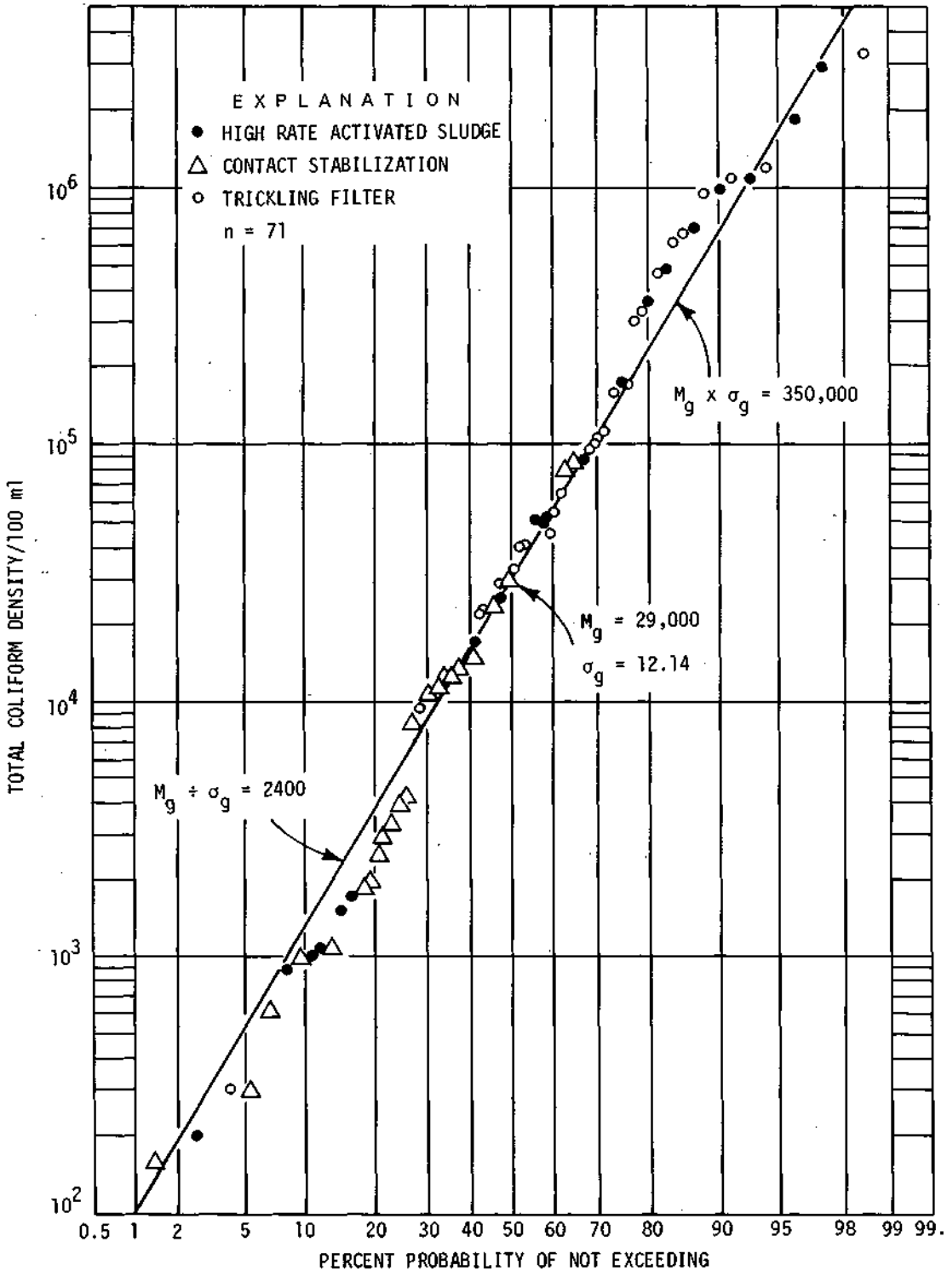

Figure 2. TC analysis by LES two-step MF membrane filter technique on samples of chlorinated effluents

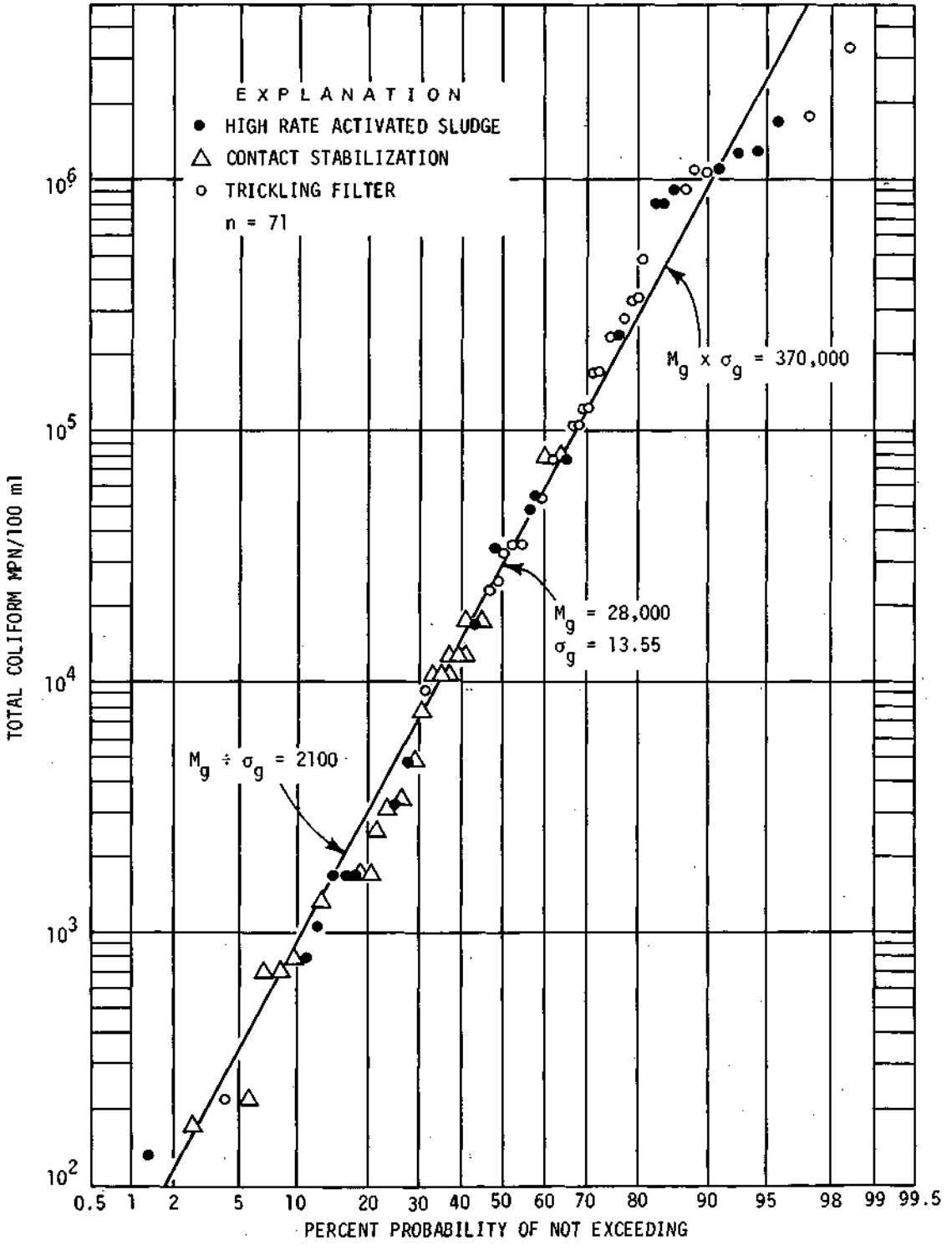

Figure 3. Total coliform MPN analysis of chlorinated effluents

LES (two-step) and Multiple-Tube Verifications. Occasionally, coliform bacteria may fail to produce colonies on membrane filters; nonconform organisms may develop sheen colonies. Verification procedures were undertaken for coliform organisms on all membrane and multiple-tube samples in accordance with procedures described by Geldreich et al.[38] The results are summarized in table 3. From 273 membrane filters, 1110 sheen colonies were selected for verification; 89.6 percent were verified as coliform organisms. Trickling filter effluent displayed the highest verification (97.5 percent) from the MF technique. It was also the highest (93.3 percent) in using the MPN procedure. From 97 MPN samples verifications ranged from 22 to 100 percent; however, 80 percent of the MPN samples reflected 100 percent verification. The average verifications for both the MF and MPN methods were higher than those reported by Geldreich et al.,[38] which were 78.1 percent for MF and 70.3 percent for MPN on samples of natural waters and sewages.

Time Effect after Dechlorination. During the course of the investigation, the question arose as to whether or not, after the dechlorination of samples, the observed bacterial densities significantly fluctuated with time. This seemed an important consideration because of the time element involved in performing comparative techniques. For this investigation, samples of three types of secondary effluent were chlorinated at varying dosages for a contact time of 15 minutes after which they were dechlorinated as previously described and kept at room temperature (20 to 22°C). Bacterial density assays were undertaken with the LES two-step and the multiple-tube methods at 15-minute intervals for a 2-hour period. The procedure not only permitted an assessment of the time element but also provided an opportunity for additional comparative analysis of the MF versus MPN techniques. The results are summarized in table 4.

There was no significant change in coliform densities during the more than 2-hour period. A comparison of the paired MF and MPN results indicates the inherent precision of the MF method over that of the MPN.

Fecal Coliforms

Comparisons for assaying fecal coliforms were made with the M-FC MF technique recommended by Geldreich et al.[16] and the confirmed MPN procedures.[4] These procedures have been accepted for fecal coliform enumerations on unchlorinated wastewater. Four chlorinated effluents were examined. One effluent, representative of the Bloomington-Normal, Illinois, sanitary district's activated sludge process, was collected from a chlorine contact tank effluent stream and immediately dechlorinated; the other three were treated with various dosages of chlorine as previously described.

The results of the two assay methods on the four effluents are shown in figure 5. It is apparent that most of the plotted

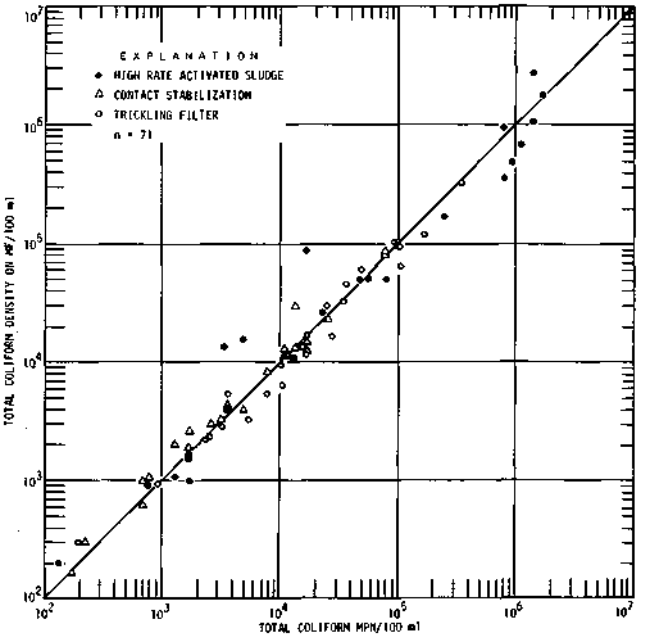

Figure 4. LES two-step MF and complete MPN results on chlorinated effluents

points lie below the line of equality. In fact, 78 are below, 12 are above, and 6 are on the line. Adjusting the equality line for MPN bias as described by Thomas[40] does little to change the pattern; 74 points are below and 22 are above the line. In several cases the discrepancy is by a factor of 10 or more which is not apparent in figure 5.

It can be concluded that the M-FC MF technique for fecal coliform detection, when applied to chlorinated wastewater effluents, is less efficient in recovery than the confirmed MPN procedure. It is suggested that an enrichment step similar to that used in the LES two-step procedure for total coliforms might improve the recovery efficiency, and further investigations seem justified.

The minimum and maximum fecal coliform ratios of MF/MPN for all tests were 0.17 and 1.46, respectively. The median ratio was 0.70. On the basis of observations from

Table 3. Validity of Two-Step MF and Confirmed MPN Tests

	MF test			MPN confirmed test			
Chlorinated effluent	Number of membranes	Number of colonies	Average percent verified	Number tested	100 percent verified	Percent verified Average	Percent verified Minimum
High rate activated sludge	66	341	86.2	27	22	92.4	46.8
Contact stabilization	101	393	88.8	35	26	91.0	50.0
Trickling filter	96	376	97.5	35	30	93.3	22.0
Total	273	1110	89.6	97	78	92.0	22.0

Table 4. Comparison of Total Coliform Density (per 100 ml) in Effluents after Dechlorination Detected by MF and MPN Techniques

Time *(min)* after dechlorination	High rate activated sludge		Contact stabilization		Trickling filter	
	MF*	MPN	MF	MPN	MF	MPN
0	1500	1700	6400	7,900	23,000	23,000
15	1500	1700	4100	6,300	21,000	35,000
30	1500	1700	4000	4,900	25,000	17,000
45	1600	1700	4000	3,300	25,000	28,000
60	1800	1700	5200	3,500	23,000	35,000
75	1500	2200	3200	11,000	24,000	35,000
90	1600	2400	3800	7,900	22,000	49,000
105	1500	2400	4500	3,500	23,000	22,000
120	1500	2200	3900	3,500	23,000	22,000
135	1500	2200			24,000	22,000
Arithmetic mean	1600	2000	4400	5,800	23,000	29,000
Geometric mean	1500	2000	4300	5,200	23,000	27,000
Median	1500	1900	4000	4,900	23,000	25,000
Mode	1500	1700	4000	3,500	23,000	22,000
Coefficient of variation, percent	6.9	15.7	21.4	46.8	5.6	33.2
Average percent verified	92.0	96.9	93.5	97.9	91.4	95.3
Range of percent verified	77.0-100	69.0-100	83.3-100	78.8-100	73.4-100	53.2-100
Chlorine dosage, *mg/l*	4.0		2.0		3.0	
Contact period, *min*	15		15		15	

* MF = LES (two-step); MPN = Completed tests

96 comparative runs, the relationship of fecal coliform densities in chlorinated effluents for the two procedures can be expressed as

$$\log MF = 0.01 + 0.94 \log MPN \quad (3)$$

The correlation coefficient is 0.99. Until a more precise procedure is developed for using MF techniques, in recovering fecal coliforms from chlorinated wastewater, a mathematical expression of this nature may be useful for estimating MPN densities. The results should be multiplied by the factor 0.851 as described by Thomas[40] for an estimate without bias.

Verification of membrane-developed colonies was made with phenol-red lactose broth and EC broth. A total of 616 blue colonies were fished for verification; 87.7 percent were verified. This was lower than the 93.2 percent verification reported by Geldreich[29] on pure cultures. .

Fecal Streptococci

Multiple-Tube versus Membrane Filter. For comparison of the MPN and MF techniques a series of FS tests on unchlorinated samples from a variety of sources were performed. The results are summarized in table 5. A statistical test of the observed data was made with the t-test of pairing observations to determine whether there is a significant difference in FS recoveries by the MPN and MF_2 methods. The results indicate there is no statistical difference in the mean values of the bacterial counts determined by the two methods (test 4 of table 9). The FS densities obtained from both procedures are comparable and probably have the same sanitary significance. Therefore the laboratory techniques of this study were considered acceptable.

One hundred and thirty-one chlorinated samples taken from three secondary sewage effluents were concurrently assayed for FS densities by the MPN and MF procedures. The colonies developed on the membrane filter that were counted after 2, 3,4, and 7 days incubation were designated MF_2, MF_3, MF_4, and MF_7, respectively. The MF_2 or MPN method is recommended by *Standard Methods.*[4]

The comparative results of MF_2 and MPN on chlorinated samples are presented graphically in figure 6. It is apparent that most of the plotted points lie below the line of equality. In fact, 107 plotted points are below, 19 are above, and 5 are on the line. From adjusting the equality line for the MPN bias, as described by Thomas,[40] most of the plotted points (97 points) are below the MPN bias reference line, 32 points are above, and 2 are on the line. Statistically significant differences were found in FS recoveries, when comparing the MPN procedure with the MF_2

Table 5. Most Probable Number and Membrane Filter Fecal Streptococci per 100 ml in Unchlorinated Waters

Source	MPN	MF_2*
Illinois River	140	64
	140	60
	130	140
Spoon River	4,600	3,100
	2,200	1,600
	790	900
	700	830
	540	900
	350	300
	280	300
	230	240
	220	200
	170	230
Spring Lake	4	4
Twin Lake	1,300	1,400
	340	420
Havana farm pond	110	75
Fiatt farm pond	1,400	1,200
High-rate activated sludge process effluent	35,000	34,000
	33,000	30,000
	27,000	30,000
	22,000	26,000
	22,000	24,000
	3,000	2,000
Contact stabilization process effluent	9,400	7,700
	4,900	5,400
	4,900	4,700
	4,600	4,300
Trickling filter process effluent	24,000	24,000
	11,000	13,000
	9,400	7,000
	4,600	5,400

* One-step M-Enterococcus agar MF count with 2-day incubation

method (test 5 of table 9). It can be concluded that the MF_2 procedure gives lower FS recovery on chlorinated effluents than does the MPN procedure. It seemed reasonable that enrichment and prolonged incubation might improve FS recovery with the MF method.

Enrichment. Azide-dextrose broth is the medium used for the presumptive test of the MPN method for fecal streptococci in waters. Brain-heart infusion broth and bile broth medium are the confirmation media of FS for the MF method. These three media were used in this study for enrichment purposes in efforts to enhance FS recovery in chlorinated effluents. The results of FS recovery on M-Enterococcus agars (MF method) with and without enrichment for chlorinated samples are shown in figures 7, 8, and 9. All FS counts in these figures were made after 2-day incubation. Although the number of colonies per filter as depicted in these figures exceeded the desirable range of 20 to 100 per filter, they were considered satisfactory for comparison purposes.

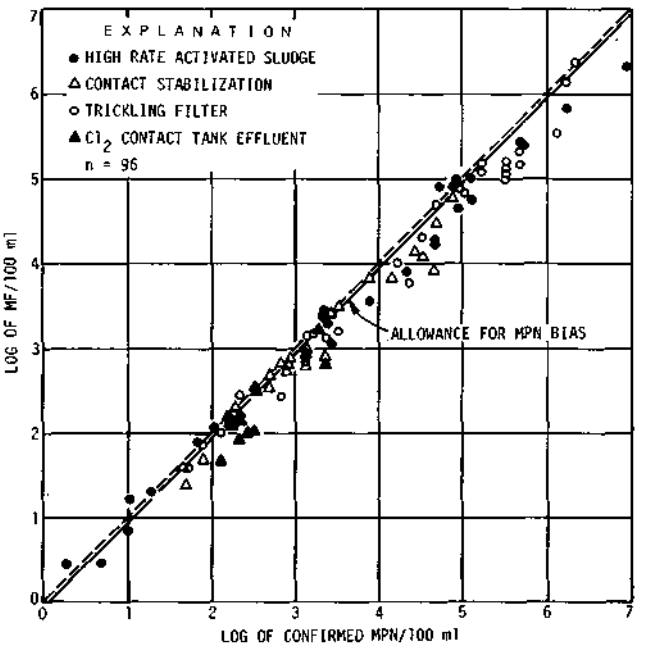

Figure 5. FC densities determined by MF and MPN techniques on chlorinated effluents

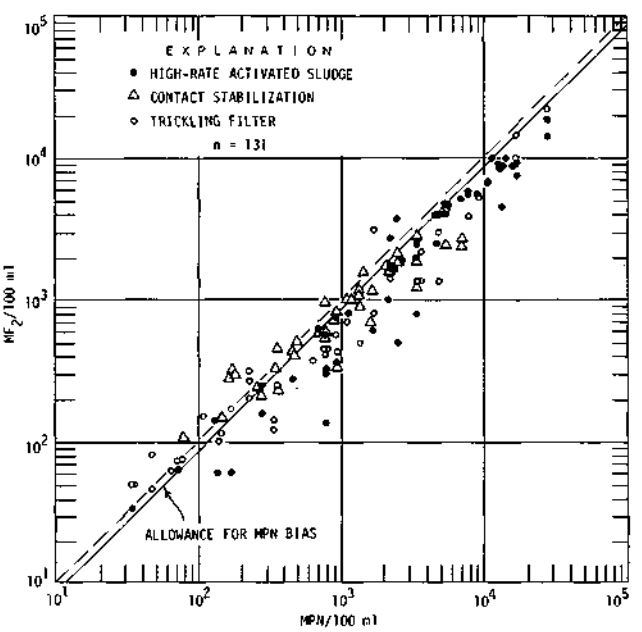

Figure 6. FS densities determined by one-step MF and MPN techniques on chlorinated effluents

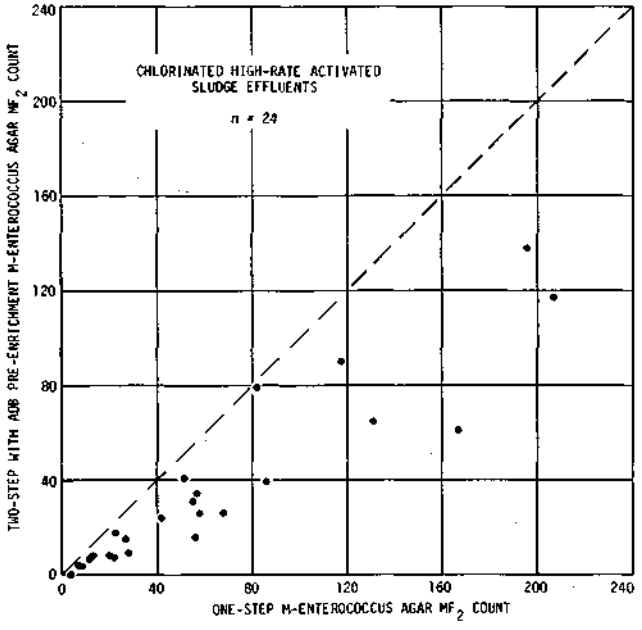

Figure 7. Comparison of FS counts on M-Enterococcus agars with and without azide-dextrose broth enrichment

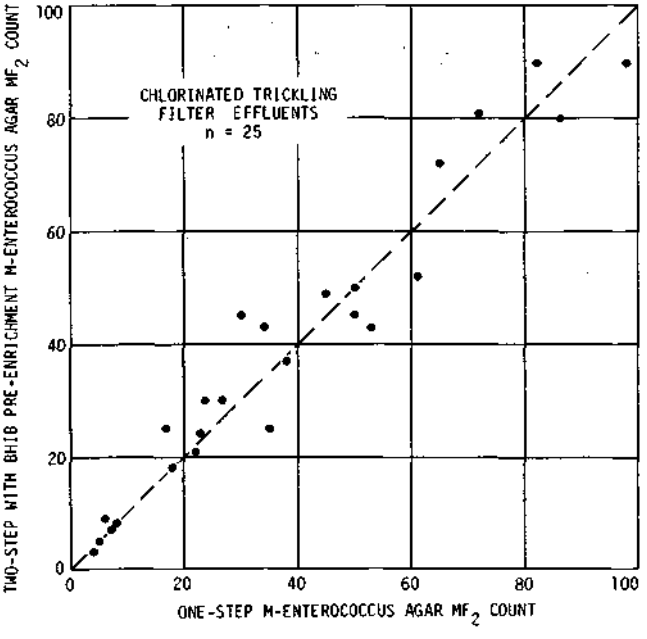

Figure 8. Comparison of FS counts on M-Enterococcus agars with and without brain-heart infusion broth enrichment

With AD broth enrichment, all plotted points lie below the equality line (figure 7). In other words, the FS recovery from chlorinated effluent on M-Enterococcus agars with AD broth enrichment falls far short of that without enrichment. This is substantiated by the t-test (test 6 of table 9) and it is concluded that enrichment with AD broth inhibits the FS recovery of chlorinated samples on membranes.

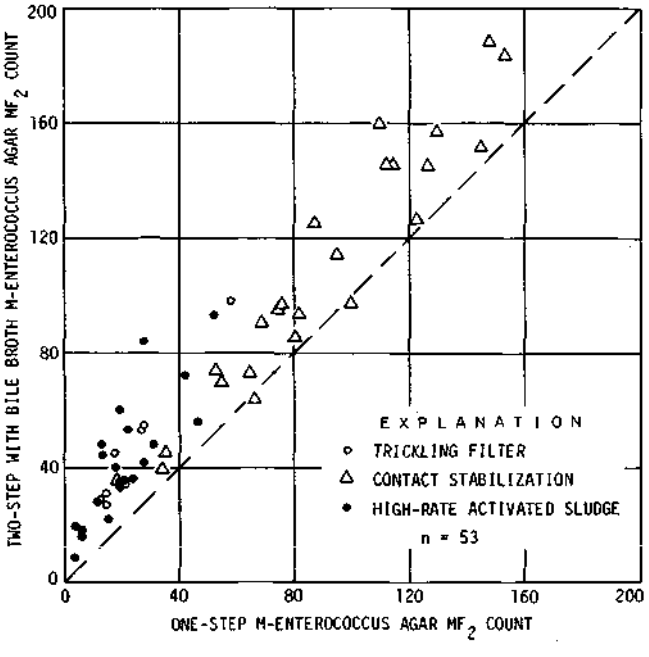

Figure 9. Comparison of FS counts on M-Enterococcus agars with and without bile broth enrichment

Figure 8 shows no appreciable difference in FS counts with or without BHI broth enrichment. Eleven plotted points lie above, 9 lie below, and 5 points are on the equality line. A statistical test (test 7 of table 9) suggests no significant difference in FS recoveries from chlorinated effluents determined by the MF method with or without enrichment. With the least square regression technique, the plotted points in figure 8 can be fitted as follows:

$$Y = 0.097 + 0.98\,X \qquad (4)$$

in which Y = FS counts by M-Enterococcus agar MF_2 with BHI broth enrichment, in organisms per 100 ml; X = FS counts by one-step M-Enterococcus agar MF_2 method, in organisms per 100 ml. The correlation coefficient is 0.97. Equation 4 shows the slope to be 0.97 with an intercept of 0.097. Thus the regression line expressed by equation 4 is almost identical with a 45-degree line. From these tests, it can be reasonably concluded there is no advantage to BHI broth enrichment for the MF method on chlorinated effluent samples.

It is quite evident from the data depicted in figure 9 that the FS recovery with bile broth enrichment is higher than FS recovery by nonenrichment techniques. Fifty-three comparisons were made on three effluents and only two effluent samples showed the enrichment FS counts slightly less than the nonenrichment. This is confirmed by statistical analyses (test 8 of table 9). It is concluded that bile broth enrichment did improve FS recovery on chlorinated effluent samples.

A peptone yeast-extract casitone enrichment broth was suggested by Rose and Litsky[36] for use with the MF method for the enrichment of FS recovery in unchlorinated wa-

ters. To determine the efficiency of the PYC broth on chlorinated effluent samples, parallel tests were made with PYC broth, bile broth medium, and without enrichment on portions of the same samples.

About one-half of the experimental results were discarded because of extremely high or low counts. The results (68 samples) where filter counts were in the desirable range of 20 to 100 are summarized in table 6. The values in table 6 represent a 2-day incubation period. For all tested effluents, with few exceptions, the recovery of ES increased with enrichment, and especially with bile broth enrichment.

The recovery ratios of enrichment to nonenrichment for each effluent are presented in table 7. The highest average ratios were 2.45:1 and 1.77:1 for bile broth and PYC enrichment, respectively. Similarly, the overall average ratios for the 68 samples were 2.14:1 and 1.60:1. The recovery ratio of PYC enrichment to nonenrichment for chlorinated samples was much less than the 2.44:1 ratio for unchlorinated waters reported by Rose and Litsky.[36]

Nineteen chlorinated samples were examined for FS densities by both MPN and PYC enrichment MF methods. The results from these assays are depicted in figure 10. The equality line was adjusted for MPN bias as described by Thomas and used for reference. Fourteen plotted points lie below the equality line, 4 are above, and 1 is on the line. A t-test analysis confirmed the differences (test 9 of table 9). From this test it is concluded that the recovery of FS from chlorinated effluents on PYC enriched membrane filters is less than that for the MPN procedure. Although prolonged incubation through 7 days on PYC enriched filters showed increasing counts with time, no attempt was made to compare prolonged PYC enriched MF counts with MPN values.

Bile broth enrichment, as mentioned earlier, gave the highest recovery of FS from chlorinated effluent samples. To compare the bile broth enriched MF_2 results with the MPN data, 45 chlorinated samples collected from three sewage effluents were subjected to FS assays, in parallel, by both methods. The results of the analyses are presented in figure 11. The ratios of the bile enriched MF_2 to the MPN FS densities were calculated, arrayed in order of magnitude, and plotted on log-probability paper. The line of the best fit was drawn. The median, or 50 percentile of the 45 ratios is 1.00. In fact, 4 ratios are equal to, 21 are greater than, and 20 ratios are less than unity. This indicates that the bile enriched MF_2 data are in very close agreement with the data obtained by MPN techniques. It was also observed that there was no significant increase in FS count on the bile enriched filters for prolonged incubation up through 7 days. It is concluded that the bile enrichment MF_2 method is superior to the PYC enrichment MF_2 method and comparable to the MPN procedure for the recovery of FS in chlorinated sewage effluents.

Prolonged Incubation.' As stated earlier, the M-Enterococcus agar MF_2 (nonenriched) technique tends to produce lower FS recovery than the MPN procedure on chlorinated effluents (see figure 6). Colonies developed for 2-day incubation were generally small. To check the effects of prolonged incubation on FS recovery for the M-Enterococcus agar MF technique all filters were counted at the end of 2, 3, 4, and 7 days incubation. Figure 12, a typical example, shows the general trend of the FS counts with incubation time. The FS recovery increased significantly up through the 3-day period. After 3 days, the FS counts leveled off

Table 6. Comparison of Recovery of Fecal Streptococci on M-Enterococcus Agars with and without Enrichment

(Chlorinated effluent, *FS/100 ml)

Sample number	Activated sludge			Contact stabilization			Trickling filter		
	NE	PYC	Bile	NE	PYC	Bile	NE	PYC	Bile
1	12	22	50	54	75	83	20	35	40
2	75	74	180	28	32	38	16	22	46
3	61	95	160	48	80	90	20	52	50
4	17	30	54	72	95	112	18	34	38
5	32	57	111	61	86	91	26	60	58
6	57	73	97	23	29	29	32	66	90
7	36	47	52	69	91	107	40	80	100
8	19	30	54	44	62	63	60	100	110
9	25	37	68	20	30	30	24	40	54
10	12	20	26	53	104	90	18	29	45
11	29	46	59	59	90	104	60	96	126
12	65	71	90	44	65	80	16	30	38
13	12	22	26	34	36	52	30	38	54
14	17	28	35	88	91	95	20	36	50
15	45	52	74	39	72	62	24	42	48
16	19	30	36	24	34	38	18	32	45
17	31	48	58	53	71	82	11	25	40
18	28	45	48	31	46	70	12	21	39
19	42	66	70	23	38	58	21	22	34
20	52	83	93	40	65	98	50	89	132
21	46	86	96	72	100	122	16	38	36
22							30	36	62
23							18	32	52
24							24	28	56
25							30	56	90
26							16	30	50
Average	35	51	73	47	66	76	26	45	61

* Incubation time was 48 hours for all cases; NE means without enrichment on M-Enterococcus agar; PYC means with PYC broth enrichment; and bile means with bile broth enrichment.

Table 7. FS Recovery Ratios of Enrichment to Nonenrichment

Chlorinated effluent	PYC/NE*		Bile/NE	
	Range	Average	Range	Average
High-rate activated sludge	0.99-1.87	1.54	1.38-4.16	2.24
Contact stabilization	1.06-1.96	1.44	1.08-2.45	1.67
Trickling filter	1.05-2.60	1.77	1.62-3.25	2.45
Overall	0.99-2.60	1.60	1.08-4.16	2.14

* Incubation time = 48 hours; NE = no enrichment; PYC = PYC broth enrichment; and bile = bile broth enrichment

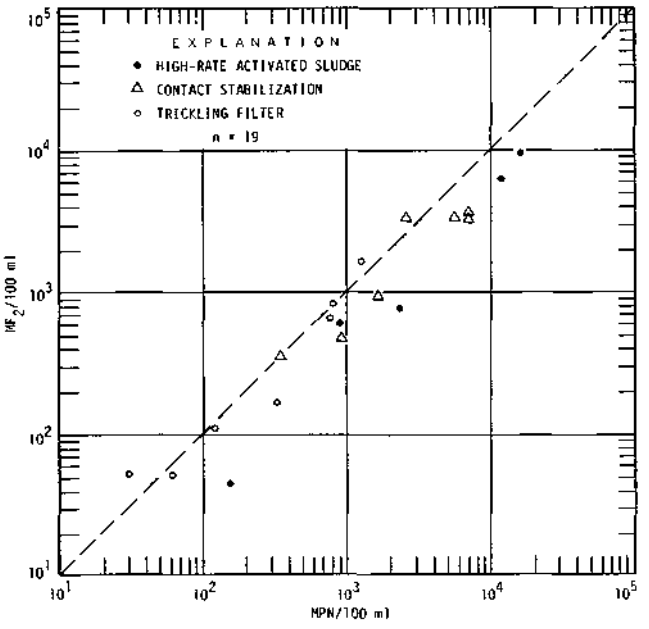

Figure 10. Comparison of FS densities determined by the MPN and the PYC enrichment MF methods

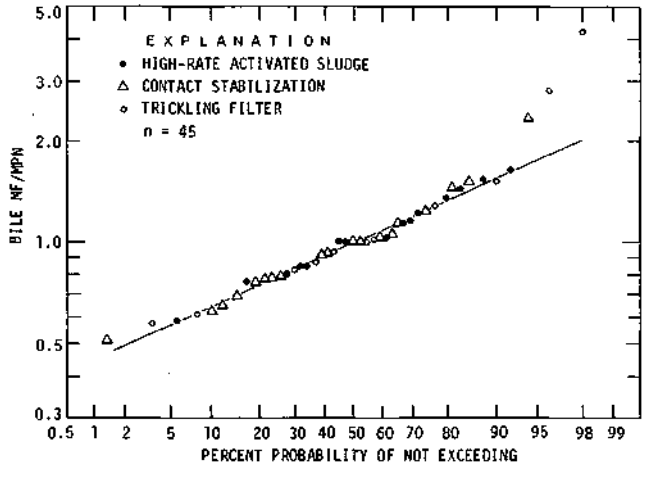

Figure 11. Analysis of FS recovery made on chlorinated effluents by the use of multiple-tube (MPN) test and M-Enterococcus agar MF with bile broth enrichment

Table 8. Fecal Streptococci Count MF_3/MF_2 Ratio

Type of effluent	Unchlorinated			Chlorinated		
	Number of samples	Range	Average	Number of samples	Range	Average
High-rate activated sludge	4	1.00-1.33	1.14	41	1.17-4.68	2.12
Contact stabilization	4	1.00-1.09	1.07	39	1.07-1.67	1.27
Trickling filter	4	1.00-1.14	1.06	44	1.07-4.28	2.07
Overall	12	1.00-1.33	1.09	124	1.07-4.68	1.84

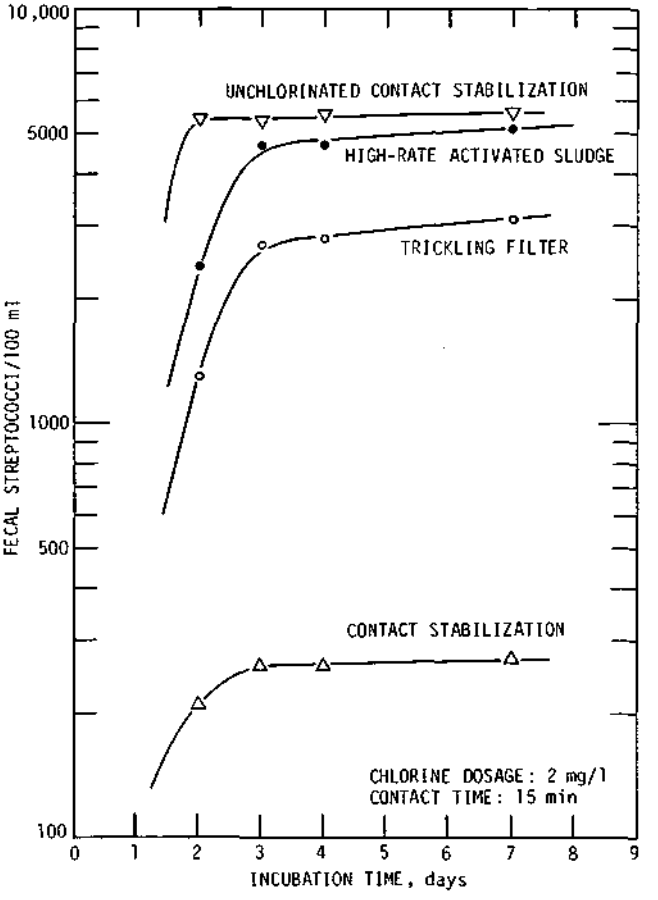

Figure 12. Recovery of FS on M-Enterococcus agar from one unchlorinated and three chlorinated secondary effluents

for chlorinated effluents. For the unchlorinated effluent sample, no significant increase was found in FS counts after a 2-day incubation. The ratios of MF_3 to MF_2 for unchlorinated and chlorinated effluents are summarized in table 8. For chlorinated samples the average MF_3/MF_2 ranged from a low of 1.27 for contact stabilization effluent, to a high of 2.12 for high-rate activated sludge with an overall average of 1.84.

Figure 13 depicts 124 comparisons of the nonenriched MF_3 data with the MPN results on three chlorinated effluents. Seventy-six plotted points are above the line of equality, and 38 are below. With the corrected MPN bias as a reference line, 101 points are above, 21 are below, and 2 are on the line. The MF_3 results were found to be slightly higher than the MPN data, especially when the FS counts were less than 500/100 ml (figure 13). For the 124 instances, the geometric mean values were 1300 MF_3/100 ml and 1100 MPN/ 100 ml. The geometric standard deviations were 3.98 and 4.93 for the MF_3 and the MPN methods, respectively. However, a statistical test (test 10 of table 9) did not indicate a significant difference between the MPN and MF_3 methods.

When comparing MF_3 and MPN results for 124 chlorinated effluent samples in a manner similar to that depicted

Table 9. Results of the t-test for Significance of Difference between Paired Observations

Test number	Compared methods	Bacteria tested	Data used	Number of observations, n	Calculated t	$t_{\alpha} = 0.05$, df*	$H_0: \mu_1 = \mu_2$†
1	MPN vs MF on unchlorinated waters	TC	Table 1	15	0.878	1.761	A
2	MPN vs LES MF on chlorinated effluents	TC	Figure 4	71	0.343	1.669	A
3	MPN vs MF on unchlorinated waters	FC	Table 1	20	1.250	1.729	A
4	MPN vs MF_2 on unchlorinated waters	FS	Table 5	32	0.135	1.696	A
5	MPN vs MF_2 on chlorinated waters	FS	Figure 6	131	6.5508	1.650	R
6	MPN vs MF_2 with ADB enrichment	FS	Figure 7	24	4.8911	1.714	R
7	MPN vs MF_2 with BHIB enrichment	FS	Figure 8	25	0.636	1.711	A
8	MPN vs MF_2 with bile enrichment	FS	Figure 9	53	11.720	1.676	R
9	MPN vs MF_2 with PYC enrichment	FS	Figure 10	19	2.724	1.734	R
10	MPN vs MF_3 on chlorinated effluents	FS	Figure 13	124	0.270	1.658	A

* Tabulated t-distribution with 95 percent confidence interval for df = n−1 (Reference 41)
† A = accept H_0; R = reject H_0

in figure 11, the median, or 50 percentile, for the MF /MPN is 1.11. Although the MF_3 values are slightly higher than the MPN data, the MF procedure for 3-day incubation on M-Enterococcus agar, without enrichment, appears applicable for the FS assay of chlorinated effluents.

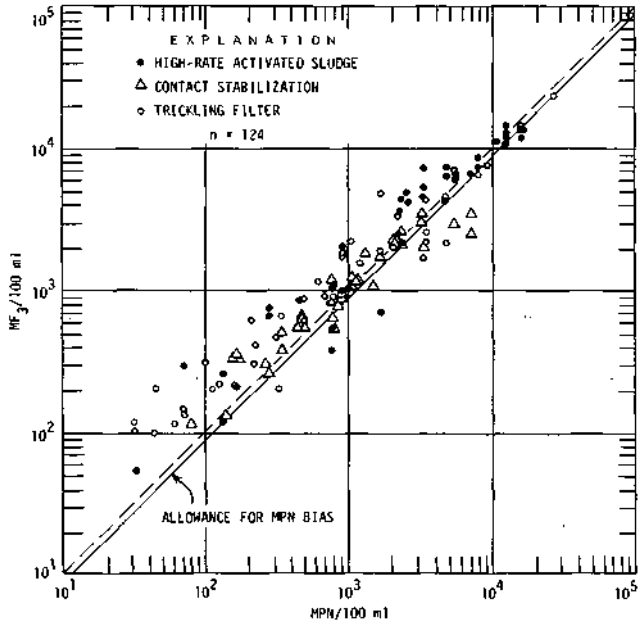

Figure 13. Comparison of FS densities determined by the MPN and M-Enterococcus agar MF_3 methods

Verification. Altogether 967 colonies were fished from 306 membrane filters and subjected to the verification procedure outlined in *Standard Methods*[4] The results of the verification are summarized in table 10. These include all colonies both with and without enrichment. After 2-day incubation, all of 688 colonies isolated from unchlorinated and chlorinated effluents were verified as fecal streptococci. Although Kenneret al.[21] reported similar 100 percent recovery of FS from the membranes for the fecal samples, Rose and Litsky[36] experienced a 94.6 percent FS verification from filters placed on M-Enterococcus agars with and without PYC enrichment for natural waters. From markings placed on the back of petri dishes during this study it was possible to distinguish 2-, 3-, and 4-day growth colonies. About 5 to 7 percent of the 3- and 4-day growth colonies were not verified as FS (table 10).

Table 10. Verification of FS Colonies

Sample	Growth after given days of incubation	Number of filters	Number of colonies	Colonies verified Number	Colonies verified Percent
Unchlorinated effluents	2	27	92	92	100
Chlorinated effluents	2	189	596	596	100
	3	74	234	223	95.3
	4	16	45	42	93.3
Overall		306	967	954	98.6

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Appendix A. Procedures of Two-Step Enrichment for Bacterial Assay

1. Prepare the enrichment medium (lauryl tryptose broth for total coliforms test; bile broth medium for fecal streptococci test) according to the label directions.
2. Rehydrate the base medium (LES M-Endo agar medium for total coliforms test; M-Enterococcus agar for fecal streptococci test) into 55 mm petri dishes in 4.6 ml amounts and allow to solidify.
3. Invert the plates containing the solidified medium and place a membrane filter absorbent pad inside on the cover.
4. Add 1.7 to 1.8 ml of the enrichment medium to each pad. Excess liquid should be removed.
5. Carefully place a membrane filter through which the water sample has been filtered top side up on the saturated pad with a rolling motion to avoid air entrapment.
6. Incubate the inverted petri dish at 35 ±0.5°C for 2 ±0.5 hours.
7. Transfer the filter from the absorbent pad to the surface of the base medium in the petri dish bottom keeping the side on which the bacteria have been collected facing upward, after the enrichment period. Total contact between the base medium and the filter should be made.
8. Discard or leave the absorbent pad in the lid and incubate the petri dish in the inverted position at 35 C for 22 ±2 hours.
9. Count the red sheen colonies on LES M-Endo agar as total coliforms; count the pink or red colonies on M-Enterococcus agar as fecal streptococci; preferably use a stereomicroscope having a light source above, and approximately perpendicular to the plane of the membrane.

Appendix B. Comparison of Total Coliform Densities Determined by the LES M-Endo Agar Two-Step MF and Completed MPN Methods

Date 1971	Chlorine Dosage (mg/l)	Chlorine Contact time (min)	TC (per 100 ml) MF	TC (per 100 ml) MPN
High-rate activated sludge effluents				
3/8	1	15	350,000	790,000
	2	15	86,000	17,000
	4	15	1,700	1,700
3/15	1	15	1,100,000	1,300,000
	1.5	15	480,000	920,000
	2	15	25,000	22,000
	4	15	900	790
3/29	1	15	690,000	1,100,000
	2	15	52,000	54,000
4/5	2	2	2,900,000	1,300,000
	2	5	970,000	790,000
	2	10	170,000	240,000
	2	15	50,000	49,000
4/12	4	5	16,000	4,900
	4	10	14,000	3,300
4/26	4	15	1,500	1,700
6/15	1	15	1,800,000	1,700,000
	2	15	50,000	79,000
	4	15	1,000	1,700
	5	15	200	130
	6	15	1,100	1,300
Contact stabilization effluents				
3/8	1	15	620	700
3/29	0.5	15	80,000	79,000
	1	15	30,000	13,000
	1.5	15	8,200	7,900
4/5	0.5	15	86,000	79,000
	1	15	14,000	17,000
4/12	2	2	11,000	11,000
	2	5	11,000	11,000
	2	10	13,000	11,000
	2	15	15,000	13,000
4/19	2	15	6,400	7,900
5/3	2	15	13,000	13,000
	3	15	4,300	3,500
Contact stabilization effluents — Continued				
5/10	2	15	23,000	24,000
	3	15	12,000	17,000
5/24	2	15	300	200
6/21	2	15	2,500	1,700
	3	15	1,000	700
	4	1	3,300	3,100
	4	2	3,000	2,600
	4	5	1,900	1,700
	4	10	2,000	1,300
	4	15	1,100	790
	4	15	160	170
Trickling filter effluents				
5/3	0.5	15	3,200,000	3,300,000
	1	15	930,000	1,100,000
	1.5	15	160,000	280,000
	2	15	64,000	110,000
	2.5	15	32,000	54,000
	3	15	22,000	23,000
5/10	0.5	15	1,200,000	1,700,000
	1	15	640,000	1,100,000
	1.5	15	110,000	130,000
	2.0	15	120,000	170,000
	2.5	15	54,000	35,000
	3.0	15	41,000	35,000
5/17	3	15	23,000	23,000
5/24	1	15	1,100,000	920,000
	1.5	15	610,000	490,000
	2	15	320,000	330,000
	2.5	15	170,000	170,000
	3	15	99,000	110,000
	3.5	15	40,000	35,000
5/26	1	15	460,000	350,000
	2	15	300,000	240,000
	3	15	100,000	130,000
	3.5	15	53,000	79,000
	4	15	28,000	33,000
	4.5	15	9,300	9,200
	5	15	300	210

Appendix C. Comparison of Fecal Coliform Densities Determined by the M-FC MF Method and the EC MPN Procedure

Date 1971	Chlorine Dosage (mg/l)	Chlorine Contact time (min)	FC (per 100 ml) MF	FC (per 100 ml) MPN
High-rate activated sludge effluents				
3/8	4	15	120	170
4/5	2	2	280,000	490,000
	2	5	45,000	94,000
	2	10	3,600	7,900
	2	15	1,200	2,700
4/12	4	5	2,300	2,300
	4	10	2,600	2,300
	4	15	2,800	2,300
6/15	4	15	160	230
	5	15	17	11
	6	15	120	110
7/12	0.5	15	250,000	540,000
	1	15	80,000	79,000
	2	15	17,000	49,000
	3	15	80	70
	4	15	20	20
	4.5	15	3	5
	5	15	3	2
7/20	1	15	19,000	49,000
	1.5	15	8,000	22,000
8/24	0.5	15	2,000,000	9,200,000
	4	1	100,000	130,000
	4	10	100,000	92,000
	4	15	80,000	54,000
	5	15	6,000	7,900
9/7	0.5	15	2,000,000	2,400,000
	1	15	680,000	1,700,000
	2	15	120,000	330,000
	5	15	56,000	130,000
Contact stabilization effluents				
3/29	0.5	15	2,600	2,600
	1	15	1,000	1,400
	1.5	15	590	790
4/5	0.5	15	7,000	7,900
	1	15	3,200	3,300
4/12	2	2	620	1,300
	2	5	730	900
	2	10	680	700
	2	15	700	790
5/3	2	15	680	790
	3	15	490	490
5/10	2	15	800	2,300
	3	15	540	790
5/24	2	15	25	50
6/21	2	15	130	170
	3	15	50	80
	4	1	350	500
	4	2	200	200
	4	5	140	200
	4	10	150	170
	4	15	140	170
	5	15	40	49
Contact stabilization effluents — Continued				
6/22	2	15	130	170
8/2	0.5	15	60,000	79,000
	0.5	15	17,000	49,000
	1	5	12,000	35,000
	1	15	13,000	28,000
	2	5	8,300	49,000
	2	15	6,700	14,000
Trickling filter effluents				
5/3	0.5	15	220,000	490,000
	1	15	20,000	33,000
	1.5	15	10,000	17,000
	2	15	1,600	3,300
	2.5	15	1,300	1,400
	3	15	1,400	1,400
5/10	0.5	15	110,000	330,000
	2.5	15	500	2,700
5/26	1	15	50,000	49,000
7/12	2	15	5,900	23,000
	2.5	15	1,500	1,700
	3	15	630	1,300
7/19	3.5	15	260	700
8/9	0.5	15	2,400,000	2,200,000
	1	15	1,400,000	1,700,000
	1.5	15	350,000	1,300,000
	2	15	130,000	330,000
8/17	0.5	15	160,000	330,000
	1	15	100,000	330,000
	1.5	15	72,000	94,000
	2	1	150,000	490,000
	2	5	150,000	170,000
	2	10	120,000	170,000
	2	15	65,000	110,000
	3	1	99,000	130,000
	3	5	72,000	79,000
	3	10	38,000	55,000
Chlorine contact tank effluents (Bloomington-Normal Plant)				
7/20	2.8	40	140	220
	4.1	50	48	130
7/26	4.3	20	650	2,400
	3.8	50	110	330
8/9	2.0	40	1,500	1,900
	2.0	50	800	1,300
8/30	2.8	40	85	220
9/13	2.8	40	320	350
	2.8	50	150	170
9/20	2.3	40	330	340
9/27	1.6	40	100	280

Appendix D. Comparison of Fecal Streptococci Densities Determined by the Standard MPN Method and the M-Enterococcus MF Method with 2, 3, 4, and 7 days Incubation on Chlorinated and Unchlorinated Secondary Sewage Effluents

Date 1973	Chlorine Dosage (mg/l)	Chlorine Contact time (min)	MPN 2-day	FS (per 100 ml) MF 2-day	MF 3-day	MF 4-day	MF 7-day
High-rate activated sludge effluents							
2/5	0		33,000	30,000	30,000		
	1	15	11,000	10,000			
	2	5	13,000	8,500	15,000		
	2	15	7,000	5,100	6,700		
	3	5	7,900	5,500	7,500		
	3	15	2,200	1,700	2,500		
	4	5	5,400	4,700	6,000		
	4	15	1,100	800	1,100		
	5	5	2,200	2,700	4,400		
	5	15	790	580	1,100		
2/12	0		22,000	26,000	27,000	33,000	33,000
	1	15	11,000	6,600	11,000	11,000	12,000
	2	5	17,000	9,000	13,000	13,000	13,000
	2	15	4,600	2,400	4,400	5,200	5,600
	2.5	5	7,900	5,800	8,800	9,100	9,400
	2.5	15	2,400	1,600	4,100	4,100	4,200
	3	5	13,000	9,000	12,000	12,000	13,000
	3	15	4,900	4,000	6,300	6,300	6,400
	4	5	3,300	2,500	5,100	5,500	5,500
	4	15	460	280	850	1,200	1,200
	4.5	15	270	250	750	900	900
2/19	0		22,000	24,000	32,000	34,000	34,000
	1	15	11,000	6,800	11,000	11,000	12,000
	2	5	13,000	8,200	13,000	13,000	13,000
	2	10	4,900	4,000	7,200	7,300	7,400
	2	15	3,300	2,400	4,700	4,700	5,100
	3	5	5,400	4,000	6,400	6,600	6,900
	3	10	3,300	2,000	3,700	3,800	4,000
	3	15	790	300	1,100	1,200	1,400
	4	5	2,400	3,800	4,900	5,000	5,000
	4	10	920	760	2,000	2,300	2,500
	4	15	280	150	670	820	870
	5	5	2,200	1,700	3,800	4,200	4,400
	5	15	70	62	290	480	730
4/23	0		3,300	2,000	2,400	2,700	2,700
	1	5	1,700	600	700	800	800
	1	15	790	130	380	400	500
	2	5	790	320	550	650	700
	2	15	130	80	120	210	210
	4	5	130	140	260	270	290
	4	15	33	33	53	60	66
5/14	0		35,000	34,000			
	1	5	28,000	19,000			
	1	15	14,000	10,000			
	2	5	28,000	14,000			
	2	10	16,000	8,500			
	2	15	9,200	5,200			
	4	5	5,400	4,600			

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Appendix D (Continued)

Date 1973	Chlorine Dosage (mg/l)	Chlorine Contact time (min)	MPN 2-day	FS (per 100 ml) MF 2-day	FS (per 100 ml) MF 3-day	FS (per 100 ml) MF 4-day	FS (per 100 ml) MF 7-day
High-rate activated sludge effluents — Continued							
6/11	2	10	17,000	7,500	15,000	15,000	17,000
	2	15	13,000	4,400	11,000	12,000	13,000
	4	10	2,400	500	2,200	2,600	3,000
	4	15	170	60	200	240	260
	5	5	920	350	980	1,200	1,200
Contact stabilization effluents							
4/2	0		4,600	4,300	4,700	4,700	4,700
	0.7	10	1,100	1,000	1,100	1,100	1,100
	0.7	20	900	730	890	900	1,000
	0.7	27	790	560	770	830	850
	1.2	5	790	600	800	800	800
	1.2	10	490	500	620	620	630
	1.2	15	490	440	520	520	540
	2	10	330	320	380	380	380
	2	15	270	210	260	260	270
	2	20	260	240	300	300	300
	3	5	170	280	320	340	340
	3	10	140	140	150	160	160
	3	15	79	110	120	120	120
4/9	0		4,900	5,400	5,400	5,600	5,600
	0.5	10	3,300	2,800	3,500	3,600	3,600
	0.5	20	2,100	1,700	2,100	2,600	2,700
	1	10	2,400	1,800	2,200	2,200	2,200
	1	20	1,700	1,200	1,800	1,800	1,800
	2	5	790	500	650	690	690
	2	15	460	420	550	570	570
	3	5	350	440	500	510	520
	3	15	180	290	340	340	330
4/16	0		4,900	4,700	5,100	5,100	5,100
	0.7	10	3,300	1,900	2,900	2,900	2,900
	0.7	20	2,400	2,100	2,700	2,700	2,800
	1.2	5	2,100	1,500	2,300	2,300	2,300
	1.2	10	2,200	1,800	2,200	2,300	2,300
	1.2	20	790	990	1,300	1,300	1,300
	2	5	1,400	1,500	1,800	1,800	1,900
	2	10	1,100	1,000	1,300	1,300	1,300
	2	15	1,300	900	1,300	1,300	1,300
	3	5	1,300	1,200	1,300	1,300	1,300
	3	15	920	810	950	980	980
	4	5	1,300	1,100	1,300	1,300	1,300
	4	15	170	320	360	360	360
6/11	0		9,400	7,700	8,400	8,600	8,700
	1	10	7,000	2,700	3,500	3,500	3,500
	1	15	5,400	2,400	2,900	3,000	3,000
	2	5	7,000	2,300	2,600	2,600	2,600
	2	10	3,300	1,300	2,000	2,000	2,000
	2	15	1,600	700	1,100	1,100	1,100
	3	10	920	330	550	620	660
	3	15	350	230	370	460	480

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Appendix D (Concluded)

Date 1973	Chlorine Dosage (mg/l)	Chlorine Contact time (min)	MPN 2-day	FS (per 100 ml) MF 2-day	MF 3-day	MF 4-day	MF 7-day
Trickling filter effluents							
2/26	0		24,000	24,000	24,000	24,000	24,000
	1	5	28,000	22,000	24,000	24,000	24,000
	1	10	17,000	14,000	15,000	16,000	16,000
	1	15	3,500	2,200	4,300	5,000	5,500
	2	5	17,000	10,000	14,000	14,000	14,000
	2	10	9,200	5,100	7,800	8,100	8,100
	2	15	3,500	1,300	2,700	2,800	3,100
	3	5	5,400	4,300	6,900	7,200	7,500
	3	10	2,200	1,400	3,200	3,400	4,100
	3	15	920	560	1,700	1,900	1,900
	4	5	1,700	3,100	4,900	4,900	4,900
	4	10	1,100	700	2,200	2,600	2,600
	4	15	350	240	640	840	1,100
3/12	0		11,000	13,000	14,000	14,000	14,000
	1	10	7,900	3,600	6,300	6,400	7,000
	1	15	4,900	3,000	4,800	4,900	5,200
	2	10	3,500	1,300	3,200	3,300	3,800
	2	15	940	420	1,800	1,900	2,200
	3	5	1,700	800	1,800	1,900	2,400
	3	10	630	370	1,200	1,300	1,600
	3	15	330	140	470	480	620
	4	10	490	420	840	800	950
	4	15	46	80	200	200	270
	5	5	220	310	600	620	670
	5	10	110	150	300	300	300
	5	15	46	46	95	100	130
3/20	0		4,600	5,400	5,800	6,200	6,200
	1	10	2,100	1,000	2,000	2,200	2,200
	1	15	790	400	800	850	950
	1	20	700	570	900	950	970
	2	5	500	400	600	600	600
	2	10	220	250	300	400	450
	2	15	170	170	220	270	270
	3	5	220	200	300	330	330
	3	10	140	120	220	250	270
	3	15	70	75	140	170	170
	4	5	70	75	150	250	250
	4	10	33	50	100	120	120
5/29	0		9,400	7,000	8,000	8,000	8,000
	1	5	4,900	1,300	2,200	2,300	2,700
	1	10	3,300	800	1,700	1,800	2,200
	1	15	1,300	500	1,600	1,700	2,200
	2	5	800	450	900	1,000	1,400
	2	10	790	450	700	720	800
	2	15	330	120	200	210	220
	3	10	130	100	200	230	270
	3	15	63	63	120	130	150
	4	10	33	90	120	120	130