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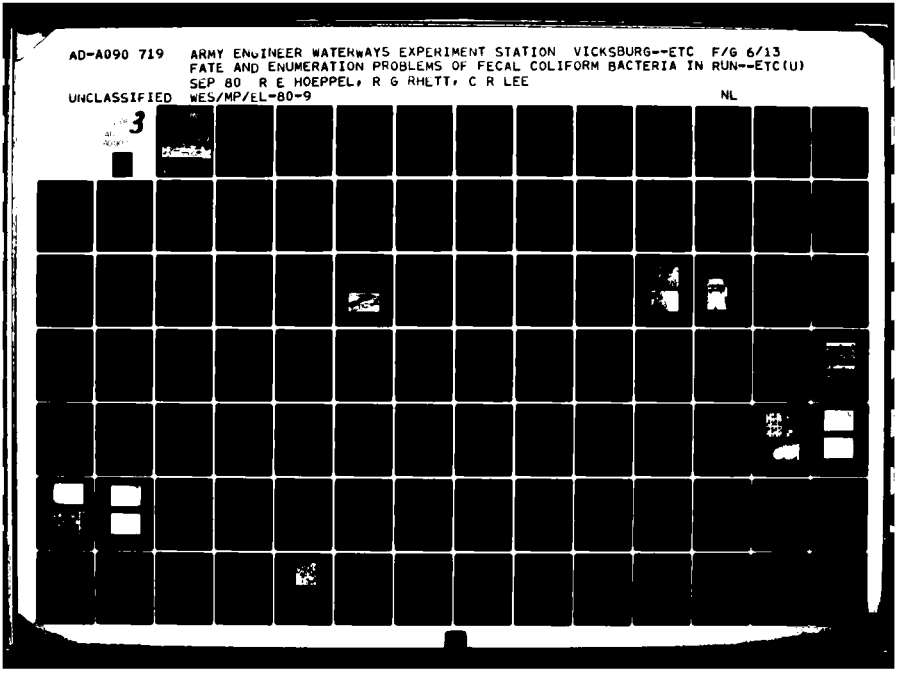

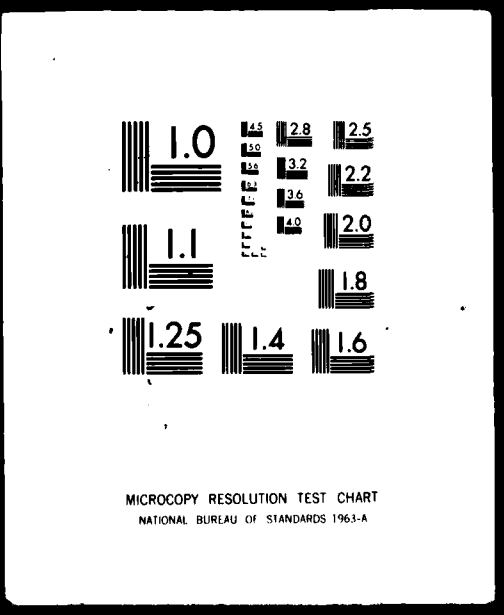

LEVEL II

TECHNICAL REPORT EL-80-9

FATE AND ENUMERATION PROBLEMS OF FECAL COLIFORM BACTERIA IN RUNOFF WATERS FROM TERRESTRIAL ECOSYSTEMS

by

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Field and greenhouse model tests were conducted, using both natural wastewater or tracer fecal coliform bacteria as overland flow system inocula. Greenhouse models contained soils previously uncontaminated or contaminated by human versus cattle fecal wastes: application rates were varied to determine best treatment conditions. The fecal coliform (M-FC) test isolates were identified to determine their origin, fate, and enumeration problems. Modifications to

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20. ABSTRACT (Continued).

the standard method for fecal coliform testing were conducted to develop improved testing methodology.

Escherichia coli was considered the best indicator bacterium for assessing recent fecal pollution and disease potential of overland runoff. The E. coli plate counts were highest in effluent runoff during the hot summer and above-freezing fall period. Enumeration problems of E. coli were less severe during the cool weather because of great reductions in interfering bacteria on test plates at this time. Field and greenhouse observations indicated that the continuously treated plots provided better conditions for protozoan predation of fecal bacteria and also for the development of a surface organic layer on the plots that aided in the removal of wastewater bacteria through filtration and entrapment. Since the greenhouse models showed mass removal efficiencies greater than 95 percent when E. coli was applied in deionized water, the inference was that organic detritus suspended in the wastewater served to protect and transport viable fecal coliform bacteria. Intermittent-flow conditions favored the growth of these bacteria on the plots.

These studies indicated that the overland flow wastewater treatment can be very efficient in removing fecal bacteria from wastewater. Methodologies that are recommended for improved removal efficiency and evaluation of test adequacy include (a) the application of wastewater to overland flow plots at sufficient rates to allow for the efficient removal of suspended solids; (b) the use of an extended (e.g. 18 to 24 hr) daily application period on the treatment plots; (c) the comparative evaluation of several stress-recovery testing procedures; (d) the use of multiple (fivefold) dilutions and low plate counts for fecal coliform testing; and (e) verification of positive bacterial colonies from seasonal samples with additional biochemical testing until the treatment system is well characterized.

SUMMARY

Fecal coliform bacteria were monitored on six field overland flow wastewater treatment plots and three greenhouse, grass-covered, overland runoff models, using the standard M-FC membrane filter test.

Wastewater was applied intermittently (0.21 cm/hr/6 hr, 5 days/week) and continuously (0.11 cm/hr/24/hr, 7 days/week) to the overland flow field plots. The greenhouse model evaluations were conducted on similar soils, previously either contaminated or uncontaminated with fecal wastes; flow conditions varied from 0.21 cm/hr/6 hr to 0.07 cm/hr/18 hr during five applications per week.

The continuously treated plots consistently showed better removal of Escherichia coli and of other fecal coliform bacteria. Field observations indicated that the continuously treated plots provided better conditions not only for protozoan predation of fecal bacteria but also for the development of a surface organic layer on the plots. This organic layer acted to increase the detention of the wastewater bacteria through filtration and entrapment. The intermittently treated plots promoted the proliferation or survival of E. coli and other fecal coliform bacteria, resulting in occasionally very high effluent counts. The greenhouse model studies, using lagoon wastewater as the influent, substantiated the preceding observations. Since the greenhouse models showed mass removal efficiencies greater than 95 percent when E. coli was applied in deionized water, the inference was that organic detritus suspended in the wastewater served to protect and transport viable fecal coliform bacteria. Intermittent-flow conditions favored the growth of these bacteria on the plots.

A 2-year, in-depth evaluation of the standard M-FC membrane filter fecal coliform test showed many interference problems when the test was used to evaluate the fecal pollution potential of overland runoff. The major interference at standard overland flow treatment rates was from the growth of bacterial colonies on the M-FC test plates, which is not representative of recent fecal pollution. Interfering colonies were most commonly fecal coliform bacteria capable of extended growth and

survival on recently uncontaminated, vegetated soils; these specifically included Klebsiella pneumoniae, Enterobacter cloacae, and intermediate forms closely related to Enterobacter. High numbers of nonlactose fermenting bacteria also caused interferences. Large numbers of interfering bacterial colonies were most common during the warm summer months when field temperatures consistently approached 35°C. Interference problems were usually avoided if total colony counts per standard-sized test plate were limited to less than 120. However, this often resulted in very low plate counts for E. coli colonies.

Escherichia coli was considered the best indicator bacterium for assessing recent fecal pollution and disease potential of overland runoff. The E. coli plate counts were highest in effluent runoff during the hot summer and the cool, above-freezing fall periods. Enumeration problems of E. coli were less severe during the cool weather because of great reductions in interfering bacteria at this time. Comparative enumerations from a two-temperature M-FC test and the M-TEC test (membrane filter tests) indicated that the very low numbers of E. coli observed in wastewater applied to the plots during the summer were caused by stress factors in the lagoon. However, the recovery of stressed cells during overland flow did not appear to be the primary cause for the very high numbers in comparable effluent runoff.

These studies indicated that overland flow wastewater treatment can be very efficient in removing fecal bacteria from wastewater. Methodologies that are recommended for improved removal efficiency and evaluation of test adequacy include (a) the application of wastewater to overland flow plots at sufficient rates to allow for the efficient removal of suspended solids; (b) the use of an extended (e.g. 18 to 24 hr) daily application period on the treatment plots; (c) the comparative evaluation of several stress-recovery testing procedures; (d) the use of multiple (fivefold) dilutions and low plate counts for fecal coliform testing; and (e) the verification of positive bacterial colonies from seasonal samples with additional biochemical testing (e.g., prepared test strips) until the treatment system is well characterized.

PREFACE

The investigation reported herein was conducted under the Department of the Army Project No. 4A161101A91D, Task 02, In-House Laboratory Independent Research (ILIR) Program, sponsored by the Assistant Secretary of the Army (R&D), and is entitled "Fate and Enumeration Problems of Fecal Coliform Bacteria in Runoff Waters from Terrestrial Ecosystems."

The work was conducted during the period November 1978 to September 1980 by the Environmental Laboratory (EL), U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The investigation was conducted by Mr. Ronald E. Hoeppel, Mr. R. Glenn Rhett, and Dr. C. Richard Lee, Ecosystem Research and Simulation Division (ERSD), EL. Dr. Charles Hagedorn and Ms. Ann Ardahl, Mississippi State University, helped with the field sampling and performed the analyses for the tracer bacterium experiments at Utica. Mr. David Bondy, EL, conducted the two-temperature incubation experiment with Utica plot sod. Additional assistance from EL personnel was provided by Messrs. Floyd Hall, Jr., Roger Brock, Thomas Sturgis, John Skogerboe, Edgar Hummert, Martin Brodie, Christopher Rockwell, and Allen Burton, and Ms. Carol Wood and Ms. Karen Preston. The study was conducted under the general supervision of Drs. Robert M. Engler, Chief, Ecological Effects and Regulatory Criteria Group, Rex L. Eley, Chief, ERSD, and John Harrison, Chief, EL.

Commanders and Directors of the WES during the conduct of this study and the preparation and publication of this report were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

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PART I: INTRODUCTION

Background

1. The U. S. Army Corps of Engineers is becoming increasingly involved in managing, monitoring, and assessing the impact of nonpoint-source pollution from land runoff to wetlands and drainage systems in the United States. The Corps' continuing Civil Works activities concerning the land treatment of municipal wastewaters and dredged material mandates interest in the quality of the discharged effluents. All civilian and Government water-based activities must consider the health aspects of water quality and regulatory agencies must evaluate the validity of present water quality indicators for establishing future criteria and standards.

2. Excessive concentrations of fecal coliform bacteria, usually greater than 200 counts per 100-ml sample, are considered an indicator of the possible presence in the natural environment of pathogenic microorganisms derived from the feces of warm-blooded animals. Considering all standard testing methods presently available, the M-FC membrane filter fecal coliform test is the most widely accepted method for determining fecal contamination of nonpotable waters. Although this test appears to be quite adequate for monitoring large bodies of water and most effluents from conventional municipal wastewater treatment facilities, the literature indicates a number of potential problems in its use for monitoring land runoff water quality. The alternate standard method, which is the multiple-tube fermentation Most Probable Number (MPN) test, overcomes some of the referenced difficulties; however, this test also suffers from interference problems. The MPN procedure also requires additional time to complete, more laboratory space, and more tedious microbiological procedures than does the standard M-FC membrane filter test.

3. The Corps' past research on overland flow treatment of municipal lagoon wastewater was conducted at the U. S. Army Waterways Experiment Station (WES) field test plots near Utica, Miss. This research included the monitoring of fecal coliform concentrations in the applied lagoon wastewater and the effluent runoff from the test plots throughout the year. Several serious problems were encountered when the standard M-FC membrane filter test was used. For example, low fecal coliform bacterial colony counts were obtained during warm summer months in influent wastewater to the overland flow treatment plots. Subsequent effluent runoff counts were often higher than influent numbers and were one to two orders of magnitude higher than levels allowable by present criteria. Another observation made at the field plots was the significantly improved removal of fecal coliform bacteria (as enumerated by the standard M-FC membrane filter test) by overland flow field test plots that continuously received lagoon wastewater 7 days/week versus those that intermittently received wastewater for only 6 hr/day, 5 days/week. Although the continuously treated plots were subjected to only half the flow rate of the intermittently treated plots at a similar 2-percent slope (2.5 cm/24 hr versus 1.25 cm/6 hr), the weekly mass addition of bacteria on the continuous plots was 2.8 times that on the intermittent plots. The factors responsible for these observations appeared to be complex and in need of further research.

Objectives and Scope

4. The preceeding observations suggested that fecal coliform testing might not be reliable for indicating the sanitary conditions on an overland flow treatment area, especially in regions with very warm summer temperatures. An in-depth study was undertaken as a result of those observations and had the following objectives:

a. To determine the mechanisms and reasons for the high fecal coliform enumerations observed in effluent runoff from the Utica overland flow site during the summer.

b. To perceive the mechanisms responsible for the better fecal coliform treatment observed on the plots receiving wastewater continuously.

c. To identify the bacteria that were being enumerated by the standard M-FC membrane filter fecal coliform test and determine if the enumerated bacteria in effluent samples are of a wastewater or an indigenous soil origin.

d. To define the conditions under which the current standard M-FC test is valid and develop a logical sequence of testing methodology that would show if the present test is valid in a given testing situation.

e. To evaluate modifications of the standard M-FC test and other rapid testing methods for their ability to improve the test reliability of fecal coliform monitoring of land runoff.

5. The need for a thorough knowledge of past research and current interpretations prompted an in-depth literature review. Field observations were then continued at appropriate time intervals while overland flow model studies were conducted in a greenhouse to simulate summer conditions during cool ambient weather, and also to allow better control over the multitude of environmental variables present in the field. Four different tests were conducted in the greenhouse: (a) application of a known fecal coliform bacterium to soil previously uncontaminated by fecal wastes; (b) addition of a biochemically tagged fecal coliform bacterium to soil previously contaminated with domestic wastewater; (c) addition of lagoon wastewater to previously contaminated soil; and (d) application of a cattle manure slurry to a pasture soil that was free from human fecal contamination. All of these soils were similar and were obtained from areas near the overland flow field test plots in Utica. Field monitoring was conducted periodically throughout the year to further substantiate the observed seasonal trends.

PART II: LITERATURE REVIEW

Water Pollution Indicator Microorganisms

Enteric bacteria

6. Although many bacterial and viral diseases are spread through ingestion of water or foodstuff contaminated by excretia, there has been considerable debate as to which microorganism or microbial group should be used to detect fecal contamination. Most bacteria that are derived from feces of warm-blooded animals are in the family Enterobacteriaceae. Many bacteria in this family are pathogens, included mainly in the genera Salmonella and Shigella. Genera that are occasional pathogens include Proteus, Yersinia, Klebsiella, and Escherichia. Other enteric bacteria are readily found in unpolluted environments, including in particular, Klebsiella and Enterobacter. The common gut bacterium of man, Escherichia coli, comprises more than 90 percent of his intestinal flora and is essential to proper colon function even though certain strains have been implicated in serious intestinal infections.[1,2,3] Because of the diverse nutritional requirements of pathogenic enteric bacteria, testing for all species or even important genera is impossible. Good testing procedures have currently been developed only for Salmonella, and the need for lengthy enrichment techniques and proper handling of pathogens negates their use as ideal indicator microorganisms.[4]

Coliform bacteria

7. At the turn of the century, the coliform group* of bacteria, including Escherichia, Klebsiella, Enterobacter, and Citrobacter, were considered indicators of water pollution. The belief that all coliform bacteria were derived from the excretia of warm-blooded animals resulted in the U. S. Public Health Service monitoring coliforms in all water sources by 1914. Thus, total coliform tests, which enumerate coliform bacteria that grow at 35°C, were developed. European researchers were

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* Gram-negative, nonspore-forming facultatively anaerobic bacilli that ferment lactose with the production of gas within 48 hr at a temperature of 35°C.[5]

simultaneously developing an elevated temperature test since E. coli, which is predominant in feces, is more selectively isolated at temperatures of 44° to 46°C. Extensive use of fecal coliform tests in the United States for monitoring pollution in nonpotable waters has been implemented only in the last two decades, even though the use of fecal coliform bacteria was emphasized several decades earlier.[3,6]

8. By current definition, fecal coliform bacteria are gram-negative, nonspore-forming bacilli that can ferment lactose at 44.5°C in 24±2 hr with gas or acid production.[7] It must be stressed that the fecal coliform tests are not totally selective for E. coli, nor are they totally selective for bacteria solely derived from warm-blooded animal excretia. However, fecal coliform tests have been found to be much more representative of fecal water pollution than are the tests incubated at a lower temperature.[3]

Other fecal microorganisms

9. Fecal streptococci, also known as the Group D streptococci, have also been extensively used to determine fecal pollution of water. These bacteria are primarily of intestinal origin and classically contain the following species and varieties: Streptococcus faecalis, S. faecalis var. liquefaciens, S. faecalis var. zymogenes, S. faecium, S. durans, S. bovis, and S. equinus. Much confusion remains concerning the relationships between the different species and water contamination problems or pathogen survival; thus, different test media promote the enumeration of different or select streptococci.[6,7] Whereas the fecal coliform bacteria (specifically E. coli) seem to predominate in human feces, the fecal streptococci seem to be derived primarily from nonhuman wastes.[6] True fecal species (e.g., S. bovis and S. equinus) have generally not been found to multiply in the natural environment and seem to die off much more rapidly than common pathogenic bacteria such as Salmonella.[7,8] In addition, these species are not indicative of pollution from human wastes.[7,9] Species that have been isolated from human feces (e.g., S. faecalis) and other biotypes that are isolated on media selective for fecal streptococci also appear to be capable of rapid growth on natural vegetation and are abundant in uncontaminated

soils.[10,11,12] Therefore, the fecal streptococci by themselves are not good indicators of pollution of natural waters or land runoff.

10. Various coliphages (viruses that infect E. coli) have recently been used to monitor water pollution under the assumption that they may have a closer correlation with pathogenic enteric virus survival in wastewaters.* However, many virologists maintain that enteroviruses and bacteriophages differ markedly with respect to their survival and removal, especially from sediment-water systems.[13,14] A recent study[14] has shown that for overland flow treatment plots containing near-neutral pH soils, the f2 coliphage showed poor sorption capabilities and a poorer removal efficiency compared with the enteric virus, Poliovirus I. Removal efficiencies for the coliphage and enterovirus ranged from 30 to 60 percent and 65 to 85 percent, respectively, on different plots. Most coliphages are negatively charged at neutral and higher pH values, which inhibits their adsorption to negatively charged clay colloids.[15]

Indicator Bacteria Ratios

Fecal coliforms:total coliforms

11. As previously mentioned, the total coliform (TC) tests are less indicative of fecal pollution of water than are the fecal coliform (FC) enumerations. However, direct fecal sampling should give comparable ratios.[8] Since E. coli usually constitutes less than a third of the coliform bacteria present in diluted raw wastewater, it is plausible that either E. coli shows a rapid die-off outside the intestinal tract or other coliform bacteria (e.g., Enterobacter and Klebsiella species) multiply rapidly and/or they are numerous in natural waters and land runoff.[3] Most FC:TC ratios for natural water sources, such as rivers and lakes, show that fecal coliform bacteria account for between 0.4 and 45 percent of the total coliform number, but are more commonly in the range of 10 to 15 percent.[4,6,16] The FC:TC ratio may roughly serve

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* Personal communication, Dr. Al Dufour, U. S. Environmental Protection Agency, Marine Field Station, West Kingston, R. I., May 1979.

to indicate pollution source but it is of questionable value since the survival of different coliform bacteria is highly dependent on environmental conditions.

Fecal coliforms:fecal streptococci

12. Ratios of fecal coliform to fecal streptococci bacteria (FC:FS) have been used to indicate whether a fecal pollution source is of human or animal origin. The initial concensus was that FC:FS ratios greater than 4 indicated pollution mainly from domestic wastewater, whereas a ratio of less than 0.6 suggested waste sources from warm-blooded animals other than man, such as domestic animals.[6,16] However, as previously mentioned, only certain fecal streptococcal species are useful as pollution indicators of surface waters, and these species correlate poorly with the survival of enteric pathogens. Thus the FC:FS ratio is of little value for water quality purposes if the contamination source is more than a day old.[8,17]

13. The FC:FS ratio has been used more recently for pasture runoff to identify the relative contributions from cattle and wildlife; ratios less than 0.05 seemed to indicate wildlife sources and ratios above 0.1 were more characteristic of grazing cattle.[18] These findings need further verification.

Fecal coliforms:enteric pathogens

14. There are many conflicting data in the literature concerning the best indicator organism(s) to use to monitor water quality. However, the general concensus is that the best indicator group for most nonpotable water sources is the fecal coliform bacteria.[3,8,19,20] Based on tests of river waters throughout the United States, Geldreich[8] noted a sharp increase in the frequency of Salmonella detection when fecal coliform densities were greater than 200 counts/100 ml of fresh water; at densities greater than 2000/100 ml, Salmonella approached a 100 percent isolation frequency.

15. Although these findings and conclusions have strongly influenced present criteria for bacterial water quality of surface waters, other data seem to refute the foregoing relationships. Studies on major Michigan rivers showed that Salmonella could be isolated when the fecal

coliform concentration was as low as 4/100 ml, but at the same time less than one in three samples with more than 200 fecal coliforms/100 ml contained Salmonella.[21] Fair and Morrison[22] showed that high-quality mountain surface water could locally contain salmonellae and related bacteria when mean total coliform numbers were 30/100 ml. Gallagher and Spino,[4] however, reported that high fecal coliform numbers do not always indicate Salmonella contamination.

16. Gallagher and Spino[4] summarized the often highly variable relationships between the numbers of fecal coliform bacteria and salmonellae. Controlled laboratory techniques showed that Salmonella typhimurium die-off was different from that of fecal coliform bacteria, although no specific identification of the fecal coliform bacteria was given; low temperatures and Salmonella regrowth were implicated in decreasing the fecal coliform to Salmonella ratios.

17. Other data[21] showed a decrease in Salmonella isolations with increasing fecal coliform numbers. This suggested that perhaps competing microbial populations in the more polluted waters were inhibiting the regrowth of Salmonella on the selective media. The close relationship between fecal coliform and Salmonella die-off was demonstrated through the use of membrane filter chambers under controlled field conditions; however, the Shigella species did show a lower rate of decline.[23] Most data thus indicate that the survival of enteric pathogens is closer to that of fecal coliform bacteria than to total coliforms or fecal streptococci,[13,16] although it is evident that different genetic strains respond differently under variable environmental conditions.[8,24]

Enteric Bacterial Survival in the Environment

18. Factors which can affect the survival or growth of enteric bacteria in soils include soil moisture content and moisture-holding capacity, temperature, sunlight, pH, organic matter or nutrients, and antagonisms or synergisms with other microorganisms.[13] Similar factors also seem to influence survival of these bacteria in natural waters.

Soil moisture and
water-holding capacity

19. Studies by VanDonsel et al.[24] indicated that high soil moisture was most important for the survival of E. coli and the growth of nonfecal coliform isolates. Warm, moist conditions were most favorable for rapid short-term growth. The relationship between soil moisture and survival time was evident from seasonal patterns: more than a 13-day survival in the wet, cool autumn and only about a 3-day survival in the dry, hot summer.

20. Fine-grained soils have been shown to elicit longer survival of fecal coliform and pathogenic bacteria because of a greater moisture-holding capacity in comparison with that of coarse-grained sediments.[13] Longer survival in clay soils may also be due to the protective effect of clay particles.[25,26] A study using clay soil columns, in contrast, showed the decline of both E. coli and S. typhimurium when the columns were saturated. This phenomenon was attributed to the effects of a changing, antagonistic microflora. However, desiccation brought about an even more rapid die-off; both bacteria survived less than 2 weeks.[27]

Temperature and sunlight

21. Cool, moist conditions generally seem to favor the survival and/or short-term growth of total and fecal coliform bacteria, whereas freezing or hot and dry conditions lessen their survival.[13,16,24] However, total agreement does not exist concerning temperature effects, probably because of the other diverse environmental variables that can interact and the problems in separating the true fecal from natural coliform populations. Some researchers[28] have noted accentuated growth and longer survival of E. coli at water temperatures exceeding 30°C, and related this better growth to temperatures similar to the 37°C temperature in the guts of most warm-blooded animals. Others have documented decreased survival with increasing ambient temperatures.[24,26,29] Jar studies of coliform bacterial growth characteristics in diluted sewage showed a shorter lag phase and a more rapid death phase at elevated temperature (e.g., 30°C versus 10-20°C). However, maximum numbers resulting from observed tenfold growth were similar at all temperatures

tested.[30] Even though repeated freezing and thawing will stress most bacteria, Salmonella and related pathogens were found to persist for extended periods under freezing conditions.[4,24] The complexity of interacting variables is evident: sunlight, moisture, and temperatures;[24,29] temperature and nutrients;[4] and temperature and its effects on the associated microflora.[24]

22. Sunlight appears to have a pronounced effect on the mortality of enteric bacteria.[13,31,32] However, the direct effects caused by ultraviolet light, rather than the associated temperature and desiccation effects, are restricted to the upper thin layer of soil, exposed surfaces of vegetation, and surface water. The interacting variables of light, temperature, and desiccation are evident, but sunlight exposure seems most important for die-off of enteric bacteria from plant surfaces.[33-35] Bell[33] summarized in his report that fecal coliforms and salmonellae have been shown to persist on vegetation for as little as 10 hours to up to a month, dependent on the degree of the negative factors of bright sunlight, high temperature, and low humidity.

Inorganic and organic nutrients

23. The occasionally longer persistence or growth of enteric bacteria in soils and sediments compared with water has most frequently been attributed to the large supply of available nutrients.[27,36,37] However, the presence of inorganic and organic nutrients in surface waters has also been implicated in the survival or short-term growth of fecal coliforms and enteric pathogens both in the water column and in subaqueous sediments.[38,39] Other studies suggest that nutrient levels above those normally present in sediments have a minimal effect on fecal coliform levels,[40] or that diverse strains react differently (i.e., positively or negatively) to elevated nutrient levels.[4] Hardness, salinity, and ionic quality of surface waters can also influence bacterial survival, although different microbial species or even strains may vary in compositional preferences.[32,41]

24. Stress, especially nutrient starvation, has been found to increase microbial sensitivity to secondary stresses such as chemical contaminants or toxins and various physical conditions. Research

indicates that most strains of E. coli, whose habitat is the environmentally uniform and nutrient-rich intestinal tract, cannot adjust to multiple stresses (e.g., starvation, nutrient addition, and a subsequent mild warming stress). Most natural microinhabitants of aquatic environments can regulate their metabolism to changing environmental conditions.[42] Thus, diverse findings under varying environmental conditions may not be unusual for fecal coliforms, since different strains of E. coli and related fecal coliform bacteria can respond in often divergent manners. The rapid die-off of fecal coliform bacteria is probably due to their inability to adjust to environmental stresses; instances of long-term survival may be due to the dominance of more resistant existing strains or favorable genetic changes in the sensitive strains. This subject will be discussed more fully in a later section.

Antagonistic microflora

25. The diversity of microorganisms in different environments undoubtedly strongly influences fecal coliform and pathogenic bacterial die-off. Although such interactions are difficult to define in soils, studies in the aquatic marine environment document microbial predation by marine bacteria and protozoa, toxification by organisms, and inability to compete with native microflora as causes for observed declines.[39] Predation of enteric bacteria by the parasitic bacterium Bdellovibrio[43] and by actinomycetes and protozoa[13,24] in freshwater environments can also be important.

Dissolved oxygen, Eh, pH, and related factors

26. Many of the mechanisms promoting the short life of E. coli and most other enteric bacteria have frequently been ill defined. The decline of E. coli in fresh and saline waters containing macrophytes and algae was experimentally shown to be related to the low carbon dioxide levels in the water produced by photosynthesis.[44] Whether the observed effect of carbon dioxide was direct or indirect, such as that promoted by the uncontrolled rise in pH or by toxic or metabolic effects resulting from decreased water alkalinity, was not discussed. Soil studies showed that very acidic peat soils (pH 2.9 to 3.7) caused the rapid decline of

both E. coli and S. faecalis, whereas raising the pH of the same soil in the range of 5.6 to 6.3 produced rapid multiplication of E. coli.[45] The indirect effects of increased nutrient availability or decreased action of inhibiting agents were mentioned.

27. Dissolved oxygen (DO) at a uniform, near-neutral pH was shown to influence the growth and die-off of total coliform bacteria in water. The coliform organisms at elevated (38 ppm) and low (0.4 ppm) DO levels showed large initial increases in numbers in comparison to low initial increases at normal DO levels (8 ppm). However, coliform bacterial die-off occurred at the lowest rate under low DO conditions.[46] An experimental lagoon study showed greater survival of total coliform bacteria at higher, more oxidized, Eh levels. This was mainly attributed to resultant changes in toxicant levels resulting from the Eh changes.[47] Experiments with E. coli placed in sterile diffusion chambers in an artifically destratified lake showed greater numbers in the anoxic bottom water in comparison to the numbers in aerobic surface water.[28] Conflicting findings and a general lack of information regarding the effects of dissolved oxygen, oxidation-reduction potential, and pH levels on survival patterns of enteric bacteria were evident from the literature.

Microbiological Characteristics of Land Runoff

Agricultural, pasture, and rangeland runoff

28. The sources for microbial pollution of runoff waters from most agricultural lands are similar to those of pastures and rangelands. The recent emphasis on the use of manure from farm animals for improving crop productivity provides for increased contamination of arable lands by enteric microorganisms. All lands used for food production or grazing are also contaminated by similar wildlife, e.g., small mammals and birds. Runoff waters from such land surfaces are considered the major sources not only for nonpoint pollution but also for all pollution of many drainage systems in the United States.[48]

29. A review article on pasture and rangeland runoff water quality[48] states that the present fecal coliform restriction is the most

difficult water quality criterion to attain for nonpoint sources. Bacterial contamination seems to be directly proportional to stocking density in pastures and rangelands but the contamination is usually short-lived with marked reductions in fecal coliform numbers only short distances downstream from examined animal concentrations.[48,49] Runoff waters from most farmlands and pastures characteristically contain excessively high total coliform and fecal streptococci counts, regardless of whether or not the area has been recently contaminated by grazing animals.[10,50-52] Fecal coliform counts exceeding the limit for bathing waters* have been reported for most grazed watersheds during storm events[49,50,52,54] and for surface drainage waters from agricultural lands.[55,56] Fecal contamination of land-surface runoff generally correlates more closely with numbers of fecal coliform bacteria than with other presently used water quality indicator microorganisms.[24,50,51] In addition, the fecal coliform group most frequently demonstrates die-off characteristics similar to the salmonellae, which is the most important group of enteric pathogens.[8,16,19,20]

Overland flow treatment
of municipal wastewater

30. Land treatment of wastewater has recently been favorably considered by both land use planners and by environmentalists because the nutrient recycling concept has been incorporated into its treatment mode, which results in replacement rather than in depletion of natural resources. However, one important concern is the contamination of soils and runoff waters with pathogens present in the wastewaters.

31. Wastewater used for land treatment generally undergoes some form of primary treatment whereby most solids and adsorbed microorganisms are previously removed. Nevertheless, many pathogenic bacteria, fungi, protozoa, viruses, and other parasites of man or animals are

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* The department of the Interior has recommended that swimming waters should contain concentrations of less than 200 fecal coliforms per 100 ml (using a log mean of all samples), and that no more than 10 percent of total samples during a 30-day period should exceed 400 per 100 ml.[53]

included in wastewaters. The number of viable or attenuated organisms is dependent on the degree of chemical disinfection, retention time before land application, and physiochemical conditions such as those mentioned in previous sections. Although pathogens have repeatedly been identified in soil systems contaminated by solid and liquid wastes, the general opinion based on reviews of research over many years is that soils tend to attenuate the virulent nature of pathogens. Land application of wastewater, when properly applied, has rarely been implicated in the direct cause of disease.[57-59] However, improperly handled wastewaters could promote a greater incidence of enteric communicable diseases.[60] Good summary articles concerning the survival characteristics of many types of enteric pathogens in soil are available to the reader desiring further information.[59,61,62]

32. Land treatment of wastewater has been categorized by the soil permeability. The overland flow mode of treatment involves poorly to slightly permeable (mostly silt and clay) soils and a crop cover that is tolerant to saturated soil conditions and prolonged flooding. Treatment in these systems emphasizes biological, especially microbial interactions, since most of the water generally flows through only the upper few centimetres of soil and organic debris. The numerous and diverse microorganisms present in the wastewater and soil are important for waste decomposition and the necessary transformation processes.[63,64] The interaction of the natural microflora has been implicated in the removal of enteric pathogens and indicator microorganisms from soils and waters.[13] However, these background populations can also cause many problems in isolation, enumeration, and identification of the indicator bacteria, including the fecal coliform group.[11]

Fate of enteric bacteria in land treatment systems

33. Most studies concerning land treatment systems for municipal wastewaters indicate good removal of enteric pathogenic and nonpathogenic bacteria. The major short-term factor for their removal is the physical filtering phenomenon, which is especially prevalent in the upper few centimetres of soil and in the surface organic mat.[65] The ionic content

and hardness of the soil solution have been reported to be important, since higher salt and divalent cation levels cause colloid instability, which results in greater bacterial adsorption.[13,65] Many studies have shown that bacteria and viruses do not travel great distances with leachates in unfractured soils and bedrock.[13] Although highly porous sandy soils have been shown to be effective in pathogen removal after the buildup of an organic stratum,[66] fine-grained sediments appear to be better for bacterial and viral retention.[13,65] However, studies using antibiotically labeled E. coli as a tracer have shown relatively rapid movement of this indicator organism under conditions of saturated flow, mainly by movement through soil macropores.[67] Such movement could also be indicative of high-rate surface runoff and overland flow conditions.

34. Factors that warrant consideration in overland flow systems include soil and vegetation retention of enteric microorganisms and their growth and/or die-off characteristics on the vegetation and soils. Vegetated land surfaces seem to be better than nonvegetated land for retaining fecal coliform and other indicator bacteria during runoff events.[51] Previous studies[11] of an overland flow system have shown a seasonal response to fecal coliform removal; notable increases in the fecal coliform numbers were detected in the effluent runoff during warm summer months, and the levels were often in excess of those in the applied influent. Such a response indicates that fecal coliform bacteria are capable of growth in the soils. Such findings have also been noted by other researchers in high-quality mountain streams contaminated with municipal wastewaters[68] as well as in pasture runoff.[18] However, large fecal coliform increases observed in very short time intervals, such as an almost fourfold increase in a 500-m stretch of a stream,[69] may be due to other factors besides bacterial multiplication. Such factors may include problems inherent in the present fecal coliform tests. These problems will be discussed in detail in Part V.

35. Although an initial growth phase may occur for fecal coliforms, a rapid decline in numbers almost always follows. Bell and Bole[69] observed that the die-off of fecal coliforms in surface soil occurred in two phases: an initial rapid phase in which 90 percent

of the bacteria died within 48 hr of wastewater irrigation, and a subsequent, slower decline over about 2 weeks for the remaining 10 percent. However, no runoff was allowed in the study, and soil desiccation probably occurred intermittently. More information is available in several good review papers concerning land treatment of wastes.[13,61,70]

36. Vegetables irrigated by the low aerosol-forming drip method, which is ideal for overland flow dispersal, showed a 38-times-higher fecal coliform count when treated with wastewater than when fresh water was applied.[71] Nevertheless, other studies[33-35] suggest that exposure of forage grasses to a few days of bright sunlight following cessation of wastewater application should eliminate fecal coliform bacteria from their surfaces. The studies stipulate, however, that different plants may have different die-off rates and rainfall can recontaminate their surfaces. Grunnet and Moller[72] noted that both S. typhimurium and coliphage showed a rapid die-off rate from mixed forage with total disappearance within 40 days. However, the coliform bacteria and E. coli, after an initial 3-day decline in counts, showed a leveling of the death rate over the remainder of the 120-day monitoring period.

Inherent Problems with Fecal Coliform Testing of Land Runoff

37. One complicating factor in monitoring overland flow treatment sites, as well as runoff from pastures and farmlands, is the continued presence of warm-blooded wildlife (e.g., birds and small mammals) and insects; these may all act to disperse uncontrollable levels of fecal bacteria.[18,50,73-76] The grazing of overland flow systems by livestock should also be considered in the interpretation of indicator bacterial counts in effluents.

38. The fecal coliform testing procedures are mainly selective for an obligate microorganism of the intestinal tract of most warm-blooded animals, namely, E. coli. However, other bacteria can grow and give a positive test reaction on the selective lactose media at an elevated (44.5°C) temperature. The most common isolates are species of Klebsiella and Enterobacter, both of which are natural inhabitants of

feces and of plants and soils in relatively contaminant-free environments. These bacteria generally constitute less than 5 percent of the fecal population.[3] They are also known to multiply in soils and on grasses because of their use of cyclitols, which are produced by many plants and persist in soil organic matter.[77,78] Although Klebsiella represented only 25 percent of the isolated fecal coliform bacteria in cool forest environments of western Canada,[79] land surfaces in warmer geographic regions of the world may induce the proliferation of these thermotolerant facultative coliforms through the processes of natural selection. The presence and growth of Klebsiella pneumoniae in the natural environment are problems, since serologically and biochemically the environmental isolates are very similar to the pathological isolates.[80,81] In one study 85 percent of the pathological strains and only 16 percent of the environmental strains were isolated by fecal coliform testing of environmental samples from the northwestern and northeastern United States, suggesting the human infectious origin of isolated colonies.[82] However, this trend may not be true for barren land surfaces in warm environments where surface soil temperatures approach the fecal coliform test incubation temperatures.[11] Since the natural strains show no epidemiologic relationship to disease,[81] more taxonomic work is obviously needed for K. pneumoniae.

39. Many bacterial isolates from fecal coliform tests are often classified as intermediate coliform types by positive or negative reaction to the standard Indole, Methyl red, Voges-proskauer,* Citrate (IMViC), or to more extensive biochemical testing. Most E. coli show a (++--) IMViC[2], whereas Enterobacter and Klebsiella are typically (+-++).[82] However, seven different IMViC patterns have been found for K. pneumoniae.[80] Other workers,[83] using lengthy biochemical testing of isolates, showed that citrate-positive variants of E. coli ((++-+)) exist among the fecal microflora of birds and domestic animals. Geldreich et al.[84] noted that an intermediate coliform type, with an IMViC of (-+-+), represented 76 percent of the strains isolated from

* A test for the formation of acetylmethylcarbinol (acetoin).

uncontaminated natural soils (251 soils from 26 states). Furthermore, using antibiotically labeled E. coli, Kasweck and Fliermans[85] noted that their strain lost the ability to ferment lactose after being subjected to heated cooling waters from a nuclear reactor plant; parallel experiments at equivalent temperatures in the absence of reactor water failed to elicit the change. Since lactose fermentation is the basis for all total and fecal coliform testing, such a change in a trait that is considered to be associated with a stable chromosome-bound genome and not with cytoplasmic genetic material (plasmids) is of great importance. Plasmid gene transfer is common among different members of the Enterobacteriaceae.[86] The transfer implies the inherent genetic heterogeneity of coliform bacteria and their ability to readily change their phenotypic expression. The need to characterize positive isolates by extensive biochemical and/or serological tests thus seems warranted.

40. Problems with the enumeration of fecal bacteria because of the growth of nonfecal bacteria on or in total coliform test media are well documented.[50,84,87] However, with increasing emphasis being placed on land runoff water quality, problems regarding accurate fecal coliform enumeration are now becoming evident.[11,79] High turbidity and high populations of natural soil bacteria can apparently interfere with the enumeration of both the total coliform and fecal coliform bacteria. The effects can be either synergistic (produce false positive counts) or antagonistic (produce false negative counts), dependent on the concentration and type of interfering solids or bacteria. True synergisms involve bacteria that would not normally produce acid and gas from lactose fermentation at the required test temperature. Fermentations of other sugars or metabolites, aided by multiple microbial interactions, can produce a positive test reaction with a nonlactose fermenter.* False negative counts may result from the desensitization of indicator bacteria by environmental stresses as well as from excessive turbidity and background microbial populations.[88] The effects of antagonisms and

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* Personal communication, Dr. Edwin Geldreich, U. S. Environmental Protection Agency, Cincinnati, Ohio, May 1979.

synergisms can affect the "presumptive" step of the MPN technique, but the problem is usually alleviated with the second (confirmed) step of this procedure for fecal coliform enumeration.[84,88] When problems with the membrane filter tests are apparent, the MPN technique is most often recommended although it also is subject to serious interference problems.[87]

Fecal Coliform Tests and Modifications

41. The American Public Health Association standard methods[5] and recommended U. S. Environmental Protection Agency (EPA) methods[7] for fecal coliform testing are very similar. Two procedures are recommended, namely, the membrane filter method using M-FC broth or agar and incubation at 44.5 $\pm$ 0.2°C for 24 hr, and the MPN method using a lengthy two-step procedure. The initial MPN step is to culture samples in lauryl tryptose broth medium at 35 $\pm$ 0.5°C; if gas forms in 24 to 48 hr, EC medium is inoculated with the culture and incubated at 44.5 $\pm$ 0.2°C for 24 hr; the presence of gas indicates the presence of fecal coliforms and the quantities are determined from statistical MPN tables.[7]

42. One of the major problems encountered with the fecal coliform membrane filter test is the antagonistic effect of environmental stresses (e.g., chlorination, high salinity, elevated temperatures, freezing conditions, nutrient deficiency, and toxic chemicals) on enumeration. These stresses often inhibit the growth or positive reaction of fecal coliforms on the test plates. Methods for overcoming stress factors are numerous, and there are many conflicting findings as to which method is best for all forms of stress.[89] Methods suggested for overcoming stressed fecal coliform bacteria and/or improving colony color include (a) the elimination of rosolic acid (a selective ingredient in the M-FC medium to help eliminate nonfecal bacteria) with incubation at 44.5°C;[90] (b) the use of a 2 percent (versus the customary 1 percent) solution of rosolic acid, with incubation at 37°C;[91] (c) the use of a two-layer technique with a nonselective enrichment medium poured over an M-FC agar layer;[92] (d) the preincubation of membrane

filter cultures at 35°C for 5 hr, followed by incubation at 44.5°C for 18 hr;[93] (e) the addition of glycerol, acetate, and reducing agents to both layers of a two-layer medium involving an enrichment overlay;[94] and (f) the addition of catalase to the surface of selective medium plates to eliminate toxic hydrogen peroxide.[95] Many of these techniques need further verification under different types of stress. Although many techniques allow better enumeration, conflicting reports have stymied inclusion of modified membrane filter techniques into the standard methods. However, modified M-FC membrane filter tests are recommended, provided that at least 10 percent of the blue colonies recovered are verified by the MPN method. If a test modification results in an increase of greater than 10 percent, then the modified method should be used throughout the study.*

43. A problem common to all fecal coliform enumeration methods is the effect of transport and storage time and temperature on recovery of coliform bacteria in collected water samples. Lonsane, Parhad, and Rao[96] showed that small declines occurred as a result of storage at both room and refrigerator temperatures, but more rapid die-off was experienced at room temperature. Storage times of up to 72 hr at refrigerator temperature showed nonsignificant differences. This is not always the case for toxic or chlorinated waters, and the EPA has devised a delayed incubation method using a holding medium containing minimum nutrients and a reducing agent.[7]

44. Other membrane filter tests have been devised in the past and were discarded mainly because of poor color differentiation or a too lengthy procedure. The newest and seemingly the best membrane filter test for selectively isolating thermotolerant E. coli is the M-TEC procedure. The test allows for the quantification of the principal enteric bacterium within 24 hr without the apparent need to subculture isolates. The M-TEC incorporates (a) a primary, selective-differential medium for Gram-negative lactose-fermenting bacteria; (b) the resuscitation of

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* Personal communication, Dr. Edwin Geldreich, U. S. Environmental Protection Agency, Cincinnati, Ohio, May 1979.

weakened organisms by preincubation for 2 hr at 35°C, followed by incubation at 44.5°C for 18-22 hr; and (c) an in situ urease test to differentiate E. coli from thermotolerant Klebsiella, which often interferes in the M-FC procedure.[97] This new test is presently being evaluated in a number of laboratories for different natural water sources.

45. Shortened tests are always being formulated because of an urgent need for rapid detection of water pollution and the need to collect and analyze samples within the time constraints of an 8-hr working day. The single-step M-TEC procedure looks promising. It adds a chromagenic cellobiose analog, indoxyl-β-D-glucoside, to the primary medium, thus eliminating filter transfer to a urease solution.[98] However, the two-step M-TEC method is considered more reliable.* Other shortened methods include a single-step MPN technique, using the appearance of growth as the sole criterion.[99] A colorimetric assay for β-galactosidase (the enzyme necessary for utilizing lactose) has also been used, based on the enzymatic hydrolysis of o-nitrophenyl-β-D-galactoside (ONPG) by fecal coliforms. The ONPG is added after a 1-hr incubation at 37°C, followed by incubation at 44.5°C until half-maximum absorbance (at 420 nm) is reached.[100] Such rapid techniques may function adequately for emergency situations but are not considered as adequate for quantitative determinations of fecal coliform bacteria.

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* Personal communication, Dr. Al Dufour, U. S. Environmental Protection Agency, West Kingston, R. I., July 1979.

PART III: MATERIALS AND METHODS

Utica Field Site

Field description

46. The field site was an experimental overland flow treatment system near Utica, Miss. The system was constructed adjacent to a 2.4-ha facultative algal lagoon, which serves to store and secondarily treat domestic raw sewage from Utica. Residency of lagoon water was estimated as 50 days. Unchlorinated lagoon water was pumped to the upslope margin of each treatment plot, where it was evenly applied across each 4.5-m-wide plot through bottom holes in a rain gutter. All plots used in this study were at a 2 percent slope, with downslope lengths of 45.5 m. The soil was a Grenada silt-loam (loess) with an artificially compacted subsoil at an approximately 15-cm depth to promote surface runoff. The predominant vegetation on the slopes was Reed canary grass (Phalaris arundinacea). A characteristic 1- to 3-cm-thick mat of organic debris and grass roots was present on the soil surface of all slopes. An aerial view of the Utica overland flow system and lagoon is shown in Figure 1.

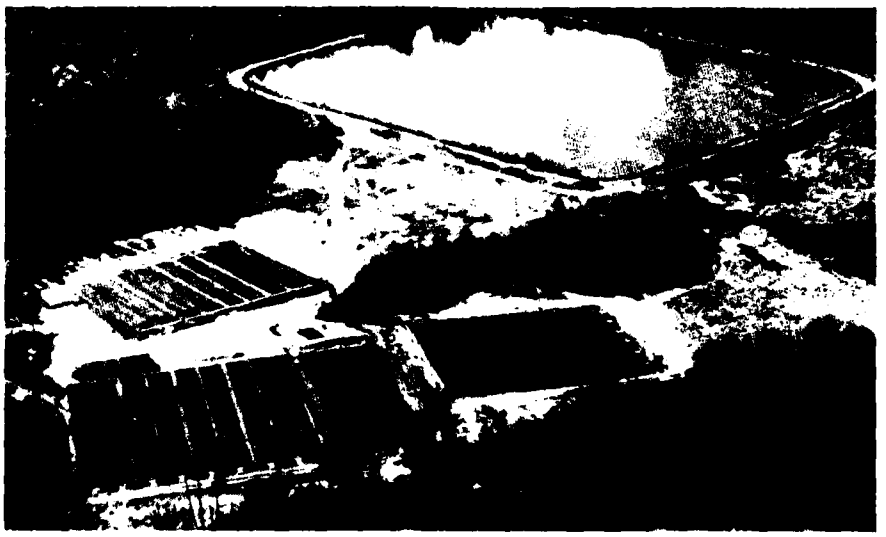


Figure 1. Aerial view of the overland flow system near Utica, Miss.

47. Lagoon wastewater was applied to the plots at two different rates: a continuous flow of 2.5 cm during 24 hr, 7 days/week, and an intermittent flow of 1.25 cm during 6 hr, 5 days/week. Experimental plots 24 and 26 received the intermittently applied wastewater from 0800 to 1400 hr, Monday through Friday. Experimental plots 23, 25, 27, and 28 received wastewater continuously. Table A1 (Appendix A) gives averages and ranges for nutrient, suspended solids, and biochemical oxygen demand (BOD) data obtained from lagoon wastewater, plot influents, and effluent runoff from the overland flow system. Additional nitrogen and phosphorus were added to the influent wastewater to raise the concentrations to approximately 20 and 10 mg/ℓ, respectively. This nutrient amendment was discontinued on 12 April 1979. The monthly pH variances for lagoon wastewater, influent, and effluent runoff (Plot 24) are depicted in Figure 2. Temperature extremes at a 1-cm depth in the test plot soil and for the air near the soil surface are shown in Figure 3. The temperature data represent 3-day averages; daily monitoring was conducted with an automatic temperature recorder/printer. Effluent discharge volumes used in the study were determined with a flowmeter during the intermittent pump-out of runoff from a concrete collection sump at the lower end of each plot.

Field sampling

48. The influent wastewater samples from Utica were collected from the application spigot for the trough at the top of each plot. Effluent samples were collected from the discharge pump at the end of each plot. Samples were routinely collected during the middle of the week after the intermittent plots were allowed to reach a saturated steady-state. Sampling times were 1100, 1200, and 1300 hr. Composites were made for each plot from the three samples taken, and a single applied wastewater composite was made to represent all plots. Samples were refrigerated at approximately 5°C and usually tested within 4 hr of collection.

49. Two bacterial tracer studies were conducted on the field plots using a strain of E. coli that was resistant to the antibiotics nalidixic acid (100 µg/ml) and sodium azide (50 µg/ml). The first test was conducted for 31 hr, from 30 October to 31 October 1979; the second

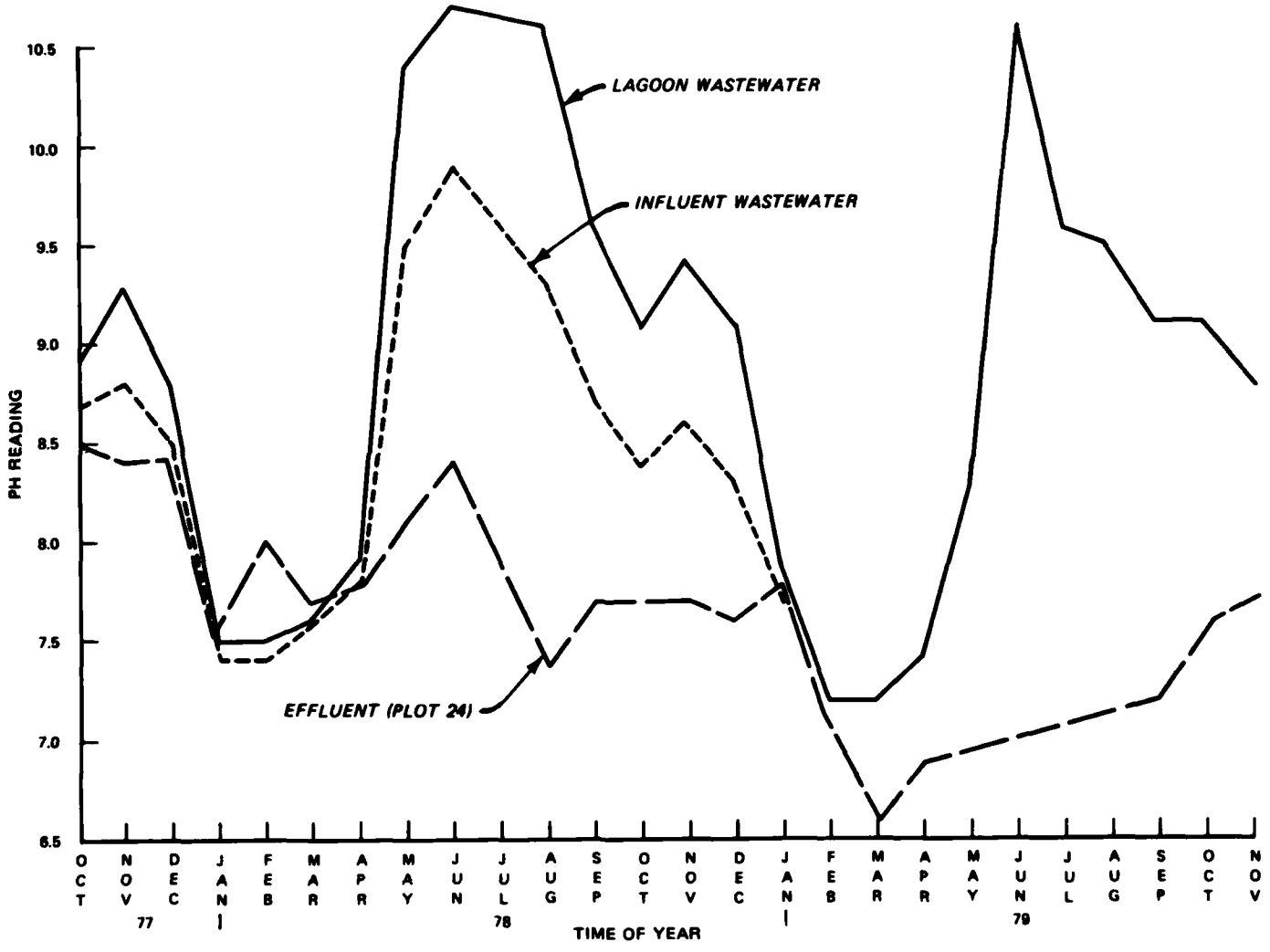
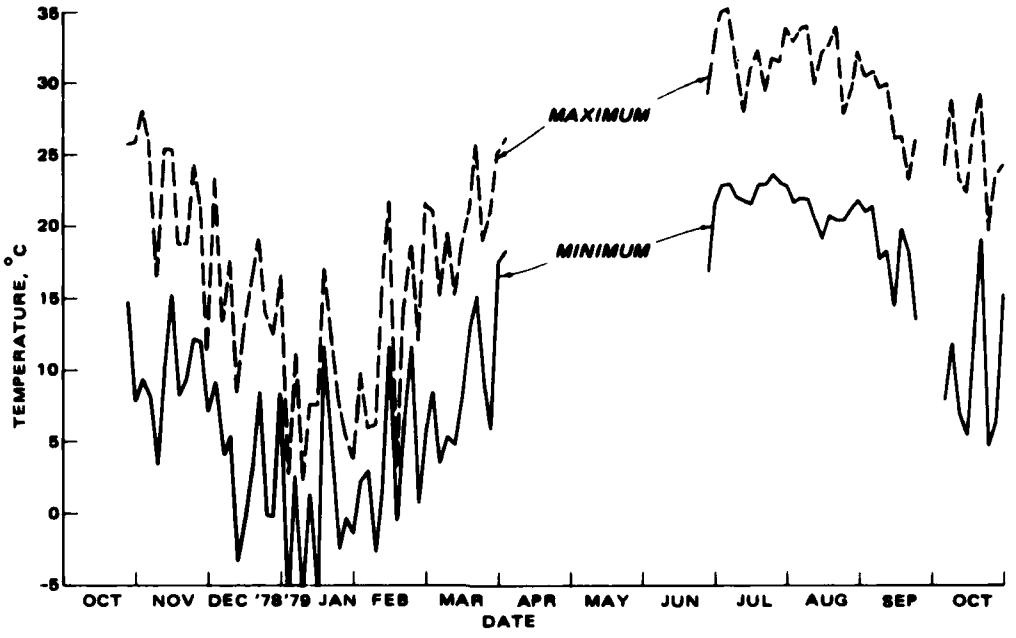
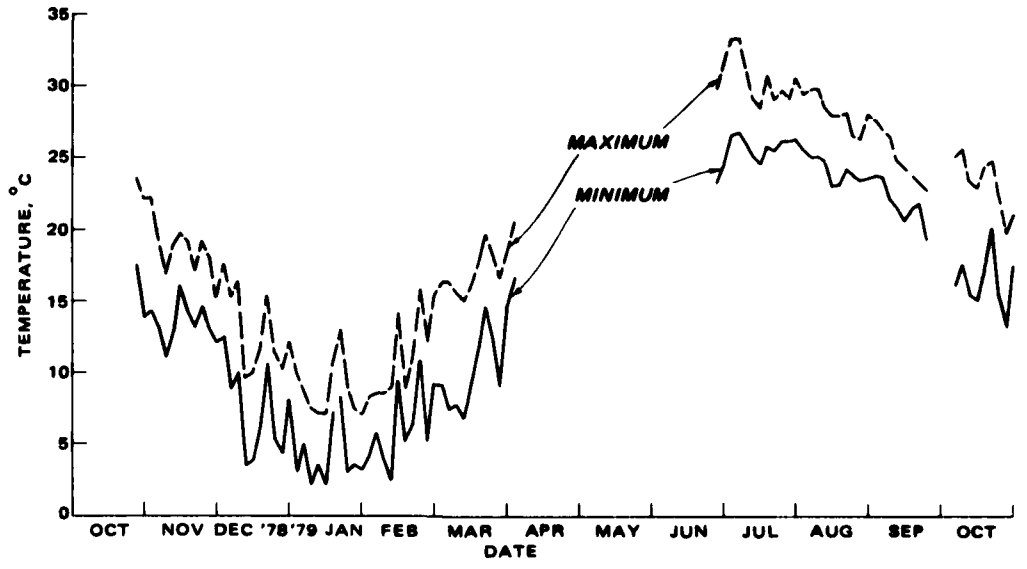


Figure 2. Seasonal pH trends at the Utica overland flow system



a. Plot soil temperature data



b. Plot air temperature data

Figure 3. Utica overland flow plot soil temperatures at a depth of 1 cm and air temperatures near the soil surface, 3-day means

test was conducted for 8 hr on 19 November 1979. The bacterium was grown in Bacto M-FC broth base for the first test and in EC broth for the second test. Each upslope plot margin was inoculated with 2 ℓ of culture; the initial and final inoculations were with 9.0×10^{14} and 6.3×10^{13} bacterial cells per plot, respectively. Samples for the first day of the initial test were collected at 15-min intervals and half-hour composites were made from 0800 to 1700 hr; second-day samples were collected hourly with no compositing, from 0900 to 1500 hr. The second test was conducted from 0900 to 1700 hr, and composites were made of two half-hour sample collections. The results of these tests were compared with previous tracer studies, using chloride ion fluctuations to assess runoff water residence times on each plot (Table A2). Pictures of plot inoculation and effluent collection of tracer bacteria at the Utica field site are shown in Figure 4.

Overland Flow Greenhouse Models

Model descriptions

50. Three greenhouse models were used to simulate the overland flow field plots. Each model was 1.2 m wide with a downslope length of 4.55 m, which gave a 1:10 slope ratio with the field plots. Each model was fitted with a vinyl plastic liner and adjusted to a 2 percent slope. Each bed was filled with field-collected grass sod, cut in 14-cm thicknesses, and packed with loose soil to fill cavities. Subsurface drainage at the downslope end of the model was provided by the installation of 3.5 m of 2.5-cm-diam plastic (PVC) pipe containing 2-mm-wide slots at 1.5-cm intervals. The pipe, constructed in a figure eight, was covered with 4 cm of fine sand and 7 cm of sod, respectively. Following its installation, the sod was statically compacted to approximate the field conditions; the sod surface lay flush with the lower lip of the models to prevent ponding. The soil beds were not seeded; a 2-month acclimation period, which allowed regrowth of the natural grasses, was provided before the experiments were conducted.

51. One model (Model 1) was filled with sod collected from

a. Inoculation

b. Effluent sampling

Figure 4. Upslope inoculation and effluent sample collection during a tracer E. coli experiment at the Utica overland flow plots

outside the treatment plots at Utica, and represented a system that was not directly contaminated at a previous time with the lagoon wastewater; the dominant vegetation was Bermuda grass (Cynodon dactylon). A second model (Model 2) was packed with sod obtained from an overland flow plot at the Utica test site. Quarter sections of the model were filled with sod collected at 0 to 3 m, 12 to 15 m, 27 to 30 m, and 42.5 to 45.5 m down the plot slope and were installed in the same respective order; the dominant vegetation was Reed canary grass. The third model (Model 3) was filled with sod collected from an actively grazed area in a cattle pasture, near the Utica overland flow system; the dominant grasses were carpet grass (Axonopus affinis) and Bermuda grass. The soil type at all collection sites was Grenada silt-loam. A view of Model 2, following grass regrowth, is shown in Figure 5. Effluent nutrient, suspended solids, pH, and temperature data for Models 2 and 3 are given in Table A3. Overland flow temperatures at different downslope distances on the models (Table A4) were obtained with a portable YSI temperature meter and probe.

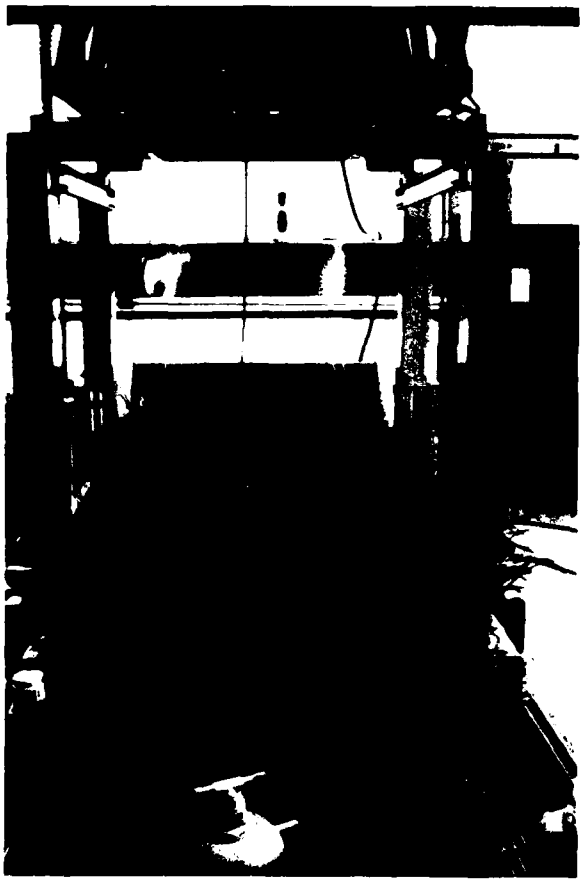


Figure 5. Greenhouse overland flow Model 2 showing the overhead sodium vapor light bank

Model application and sampling

52. The three models were usually run at the same flow rate, namely, 0.21 cm/hr for 6 hr, 5 days/week, with a weekend drying period. Deionized (reverse osmosis)

water was applied during the acclimation period and as an influent for all but one study. For the first study, adding a morphologically distinct E. coli strain to Model 1, a standard methods broth was inoculated. For the second study, adding a streptomycin-resistant strain of E. coli to Model 2, 740 mg of streptomycin was dissolved in distilled water, filter sterilized, and added to 1 ℓ of standard methods medium. The medium was cultured for 24 hr at 37°C, and a 1- to 5-ml aliquot was usually added to 70 ℓ of deionized water in the influent storage bucket. Deionized water was also used to leach cattle manure (3 g dry weight), which was added as a slurry to the upper third of Model 3 twice each week. Wastewater was applied to Model 2 during a fourth experiment at 0.21 cm/hr/6 hr, 5 days/week; 0.07 cm/hr/24 hr, 5 days/week; 0.14 cm/hr/24 hr, 5 days/week; and 0.21 cm/hr/24 hr, 5 days/week. The wastewater was collected from the lagoon every Monday and stored in the greenhouse for the duration of the week's application.

53. Influent was pumped from the application bucket through vinyl plastic tubing to a Plexiglas tray across the upslope end of each model with a peristaltic pump. Effluent runoff was collected in a tray at the downslope end of each model and distributed through vinyl plastic drainage tubing to between three and five 20-ℓ polypropylene jugs, which served to monitor daily runoff volumes. Influent sample collection was from the pump discharge tubing, whereas effluent sampling was usually from the tubing that drained the downslope collection tray. Samples were stored at 5°C until analyses were performed.

54. Greenhouse temperatures were usually kept uniformly at 20°C at night and 29.5°C during the day. However, the temperature was allowed to climb as high as 38°C during the fall of 1979 to simulate maximum field temperatures recorded during the summer. A high-intensity sodium vapor light bank was used on Model 1 and intermittently on Model 2 (Figure 5) to simulate summer light intensity and duration. These lights possess the wavelengths needed for good plant growth.

Laboratory Procedures and Equipment

Sample preparation and analysis

55. Samples were tested for fecal coliform bacteria usually within 4 hr after collection, although overnight storage at 5°C was occasionally allowed. The samples were tested both undiluted and diluted. Dilution blanks consisted of a sterile 99-ml, 0.25-Molar phosphate buffer, pH 7.2. Tenfold dilutions were commonly made to 10^{-2}, although fivefold dilutions were used when high background colony interference was prevalent. Occasionally, 50-100 ml of an undiluted sample was needed to obtain a valid fecal coliform count.

56. The samples were then analyzed for fecal coliform bacteria by the M-FC membrane filter technique, according to the standard methods.[5] Tracer bacteria, resistant to nalidixic acid and sodium azide, were enumerated on M-FC agar containing 100 mg nalidixic acid and 50 mg sodium azide per 1 ℓ of medium. Most filtration was with Millipore 0.45-µm pore size, type HAWP filters (Millipore Corp., Bedford, Mass.); some filtration was performed with Gelman 0.45-µm pore size, GN-6 filters (Gelman Instrument Co., Ann Arbor, Mich.). Undiluted samples were added to a 50-ml phosphate buffer in the filter funnel prior to filtration. A constant temperature bath (Blue M Electric Co., Blue Island, Ill.) was used for membrane filter plate incubation.

57. Samples were also occasionally analyzed by the standard methods MPN procedure,[5] the one-step[98] and two-step[97] M-TEC tests, and by a modified (two-temperature) M-FC test[93] for comparison with the standard M-FC test results. The one-step and two-step M-TEC test media and analytical procedures are given in Tables A5 and A6, respectively. The M-TEC test plates were sandwiched between two 5-cm-thick layers of polyethylene foam and incubated for 20-24 hr at 44°C in an air incubator.[98] The modified M-FC test plates were preincubated at 35°C for 3-4 hr in an air incubator and were then transferred to the water-bath for an additional 18- to 20-hr incubation at 44.5°C.

Fecal coliform
enumeration and identification

58. Following the proper incubation period, the plates with valid colony numbers were examined and counted under a dissecting microscope, usually at the 10X setting. Representative colonies of various color combinations and morphological characteristics were streaked onto selective coliform media (EMB and McConkey Agar),[7] Gram stained, and inoculated onto API-20E biochemical test strips (Ayerst Laboratories, Inc., 200 Express St., Plainview, N. Y. 11803), which are designed to identify members of the Enterobacteriaceae and certain other gram-negative bacteria.

59. Photography was used to document various blue colonies for future counting purposes and long-term comparisons. Pictures of individual colonies were made using a 35-mm camera mounted on a Bausch and Lomb dissecting scope. Pictures of the membrane filter plates were made in open shade with a macroadjustable lens on a 35-mm camera. Tungsten 160 film was used in the camera mounted on the dissecting scope and Kodacolor II film was used for the photographs of test plates.

Physicochemical testing

60. Nutrient (total Kjeldahl nitrogen, ammonia, nitrate, total phosphorus, and orthophosphate) determinations were made with a Technicon Auto Analyzer II according to EPA recommended procedures.[101] Suspended solids were determined according to the EPA procedure for nonfilterable residue,[101] using Millipore (47-mm diameter) microfilter glass filters. The BOD measurements were performed according to standard methods.[5]

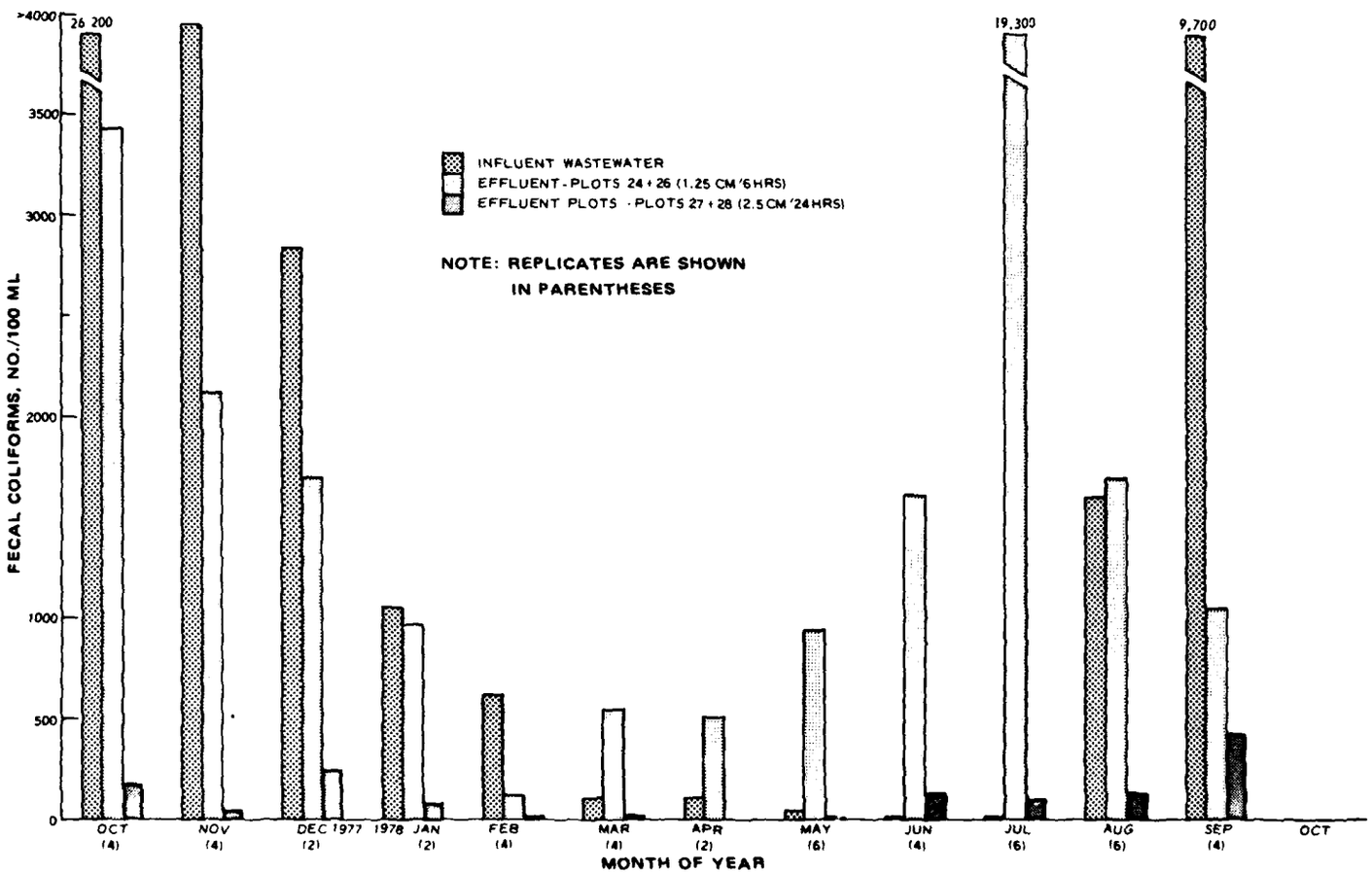
PART IV: RESULTS

Utica Overland Flow Field Plots

61. Enumerations of fecal coliform bacteria in influent (algal lagoon wastewater) and effluent runoff from grass-covered overland flow treatment plots near Utica, Miss., were conducted routinely from 1976 through 1979. The standard M-FC membrane filter test was used to enumerate blue bacterial colonies as fecal coliforms. Results through August 1977 have been published elsewhere.[11] The bar graphs in Figure 6 depict average monthly fecal coliform enumeration data, collected between October 1977 and October 1979. Figure 7 shows these same data combined as monthly averages. Effluent data for Figures 6 and 7 have been separated to depict average fecal coliform counts from each set of two plots run at different daily wastewater application rates, namely, 1.25 cm in 6 hr on 5 days (intermittent application) and 2.5 cm in 24 hr on 7 days (continuous application).

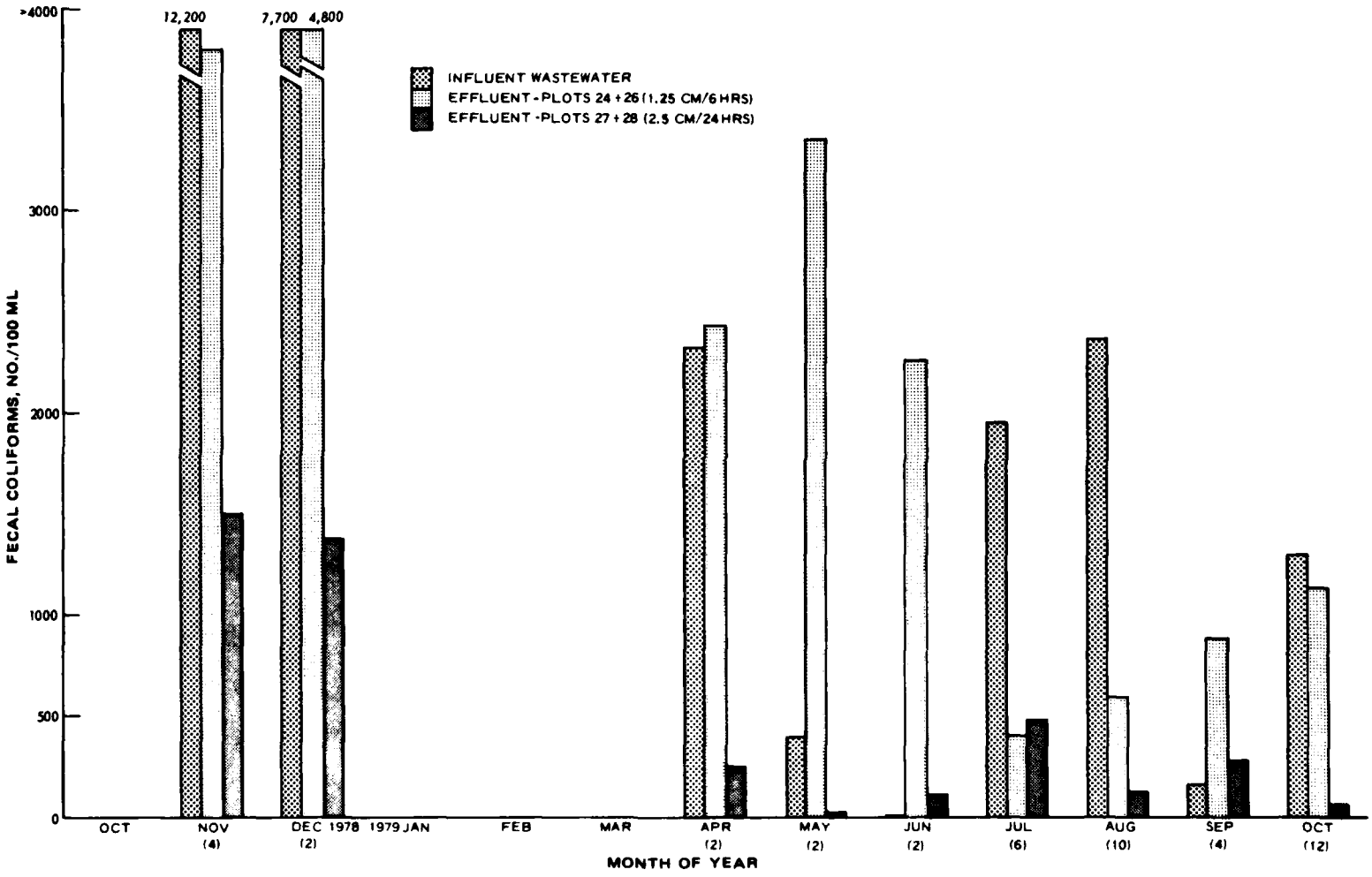
General observations

62. Fecal coliform data obtained during the first half of the four-year monitoring program at Utica showed very high counts in effluent samples collected during the late spring and summer months.[11] The more complete and more reliable data collected for this study (Figures 6 and 7) show high effluent counts of greater than 1000 per 100-ml sample from April to December. The lowest effluent numbers were obtained during the cold late-winter months of 1978 (Figure 6). Four years of monitoring also showed that the overland flow plot effluents frequently gave much higher fecal coliform counts than comparable influent (lagoon wastewater) samples during the warm summer months of May, June, and July. Statistical analysis of daily test data during 1978 and 1979 (Appendix B, Table B1) showed that most of the excessive effluent peaks depicted in Figure 6 are caused by a few very high measurements with a wide daily variability. The very high values were included in the data analysis only when acquired from plates with low colony counts, e.g., less than 100 per plate.



a. October 1977-October 1978

Figure 6. Fecal coliform counts in Utica overland flow plot samples receiving intermittent or continuous wastewater at different flow rates (Continued)



b. October 1978-October 1979

Figure 6. (Concluded)

63. Influent wastewater data (Figures 6 and 7) showed that the highest fecal coliform counts occurred during October and November. The cool weather in the fall should have prolonged the survival of fecal coliform bacteria[24] while the accompanying low rainfall provided negligible dilution of the lagoon wastewater. Declining fecal coliform counts were encountered in the plot influents (i.e., lagoon wastewater) from January to July (Figures 6 and 7). This decline appeared to be caused by two separate phenomena. Decreased counts during the winter and early spring (January through April) probably resulted from a dilution effect produced by high seasonal rainfall and from a slow overturn of lagoon water created by the higher density of cooler surface water. Minor quantities of land runoff enter the lagoon since it is not situated on a natural drainage system. Thus the rainfall dilution would mainly be direct. An overturn phenomenon would tend to bring more aged bottom lagoon water up to the near-surface intake pipe for the overland flow plots. The almost nil fecal coliform bacteria enumerated in influents during the summer months (May through July) appeared to be caused by an elevated pH (i.e., greater than 9.5) in the algal lagoon water (see Figures 2 and 6). The high pH effect was confounded by other stress factors, including algal blooms (e.g., high nutrient status and presence of algal toxins) and elevated temperature. Multiple stresses are generally considered deleterious to some fecal coliforms such as E. coli.[42] Consequently, the reported influent wastewater counts on the highly selective M-FC membrane filter medium may not have been representative of the viable count due to poor recovery of stressed cells. The release of ammonia from dying algae may have also stressed the fecal coliform bacteria since at pH values above 9, a large proportion of the ammonia is in the highly toxic, unionized form. Ammonia toxicity has been considered to increase the die-off rate of coliform bacteria.[102] A two-temperature incubation modification of the M-FC test[93] and the two-temperature, two-step M-TEC test[97] were performed to test for stress. These findings will be discussed in a later section.

64. Relatively low and declining fecal coliform counts in overland flow plot effluents from January to March may have been due to

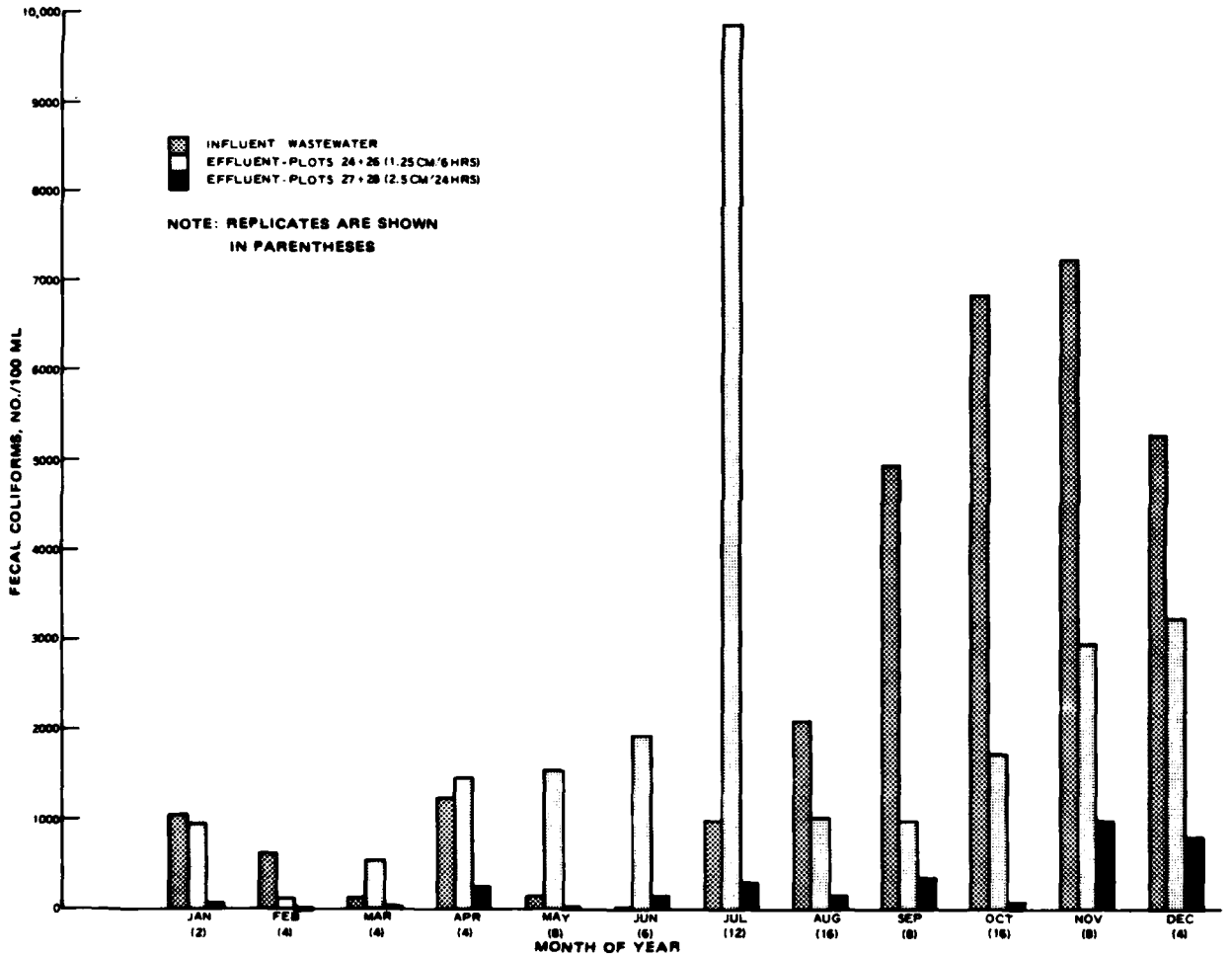


Figure 7. Monthly average fecal coliform counts in Utica overland flow plot samples

die-off or stress induced by continual freezing and thawing at or near the soil surface of the overland flow treatment plots at that time of year (see Figures 3 and 6).[13] In the previous study[11] the lowest effluent fecal coliform numbers were also observed during the winter months. The occasionally very high and sporadic effluent fecal coliform counts encountered during summer monitoring were probably caused by several factors: rapid fecal coliform growth and decline; indigenous soil bacteria giving a positive response on the M-FC membrane filter medium; and false positive soil bacteria that produce positive test reactions through synergistic interactions. These phenomena will be discussed in greater detail in Part V.

Flow rate effects

65. A significant difference ($P \leq 0.05$) was found between fecal coliform counts obtained for effluents from the intermittent-flow plots (Plots 24 and 26, 1.25 cm/6 hr/5 days) versus the continuous-flow plots (Plots 27 and 28, 2.5 cm/24 hr). This is clearly evident from a comparison of the two effluent bar graphs depicted for each month in Figures 6 and 7, and perusal of the raw data in Table B1. Great emphasis was placed on determining the reasons for the apparently better fecal coliform removal by the continuous plots. Although the continuously applied wastewater was delivered at half the flow rate of the intermittently applied influents, both the weekly wastewater volume and the bacterial mass loading were 2.8 times greater for the continuous-flow plots. Additionally, the intermittent-flow plots showed a greater than elevenfold increase in counts when compared with the continuous-flow plots. The intermittent-flow plots gave only a 25 percent decrease in fecal coliform counts compared with the influent levels. The continuous-flow plots, in comparison, elicited a greater than 93 percent removal of the influent fecal coliform counts. Mass removal efficiencies for the intermittent and continuous wastewater applications approximated 44 and 90 percent, respectively, based on average monthly volumes for the testing period.

Tracer studies with E. coli

66. A genetically marked strain of E. coli, resistant to both nalidixic acid and sodium azide, was used to inoculate influents to six

overland flow plots at Utica, Miss. Two sampling periods were involved. The initial monitoring period, from 30 to 31 October 1979, was over a 31-hr period (Figure 8), whereas the second monitoring period, on 19 November 1979, was over an 8-hr period (Figure 9).

67. The first monitoring involved the addition of 9.0×10^{14} total E. coli cells to the upslope soil margin of each of four plots. This inoculum was then leached downslope with lagoon wastewater and applied at two different flow rates as follows:

Plot 24 - Intermittent flow: 0.21 cm/hr, 6 hr/day, 5 days/week

Plot 26 - Identical to Plot 24

Plot 27 - Continuous flow: 0.11 cm/hr, 24 hr/day, 7 days/week

Plot 28 - Identical to Plot 27

Since these plots were run in an identical manner to previous monitoring activities (1976 through 1979), the use of the tracer bacterium accurately depicted the functioning of each plot during the time period of the study. Specific questions include (a) the rate of initial fecal coliform movement down each 2 percent plot slope (plug flow); (b) the latent effects caused by bacterial adsorption in the overland flow systems; (c) the rate and nature of bacterial die-off or growth on the plots; and (d) the equivalency of replicated plots.

68. The second monitoring involved the addition of 1.3×10^{13} total E. coli cells to the upslope soil margin of each of six plots. Several changes in flow rates and duration of operation were made for different plots as follows:

Plot 23 - Continuous flow, acclimated for 6 weeks: 0.11 cm/hr, 24 hr/day, 7 days/week

Plot 25 - Identical to Plot 23

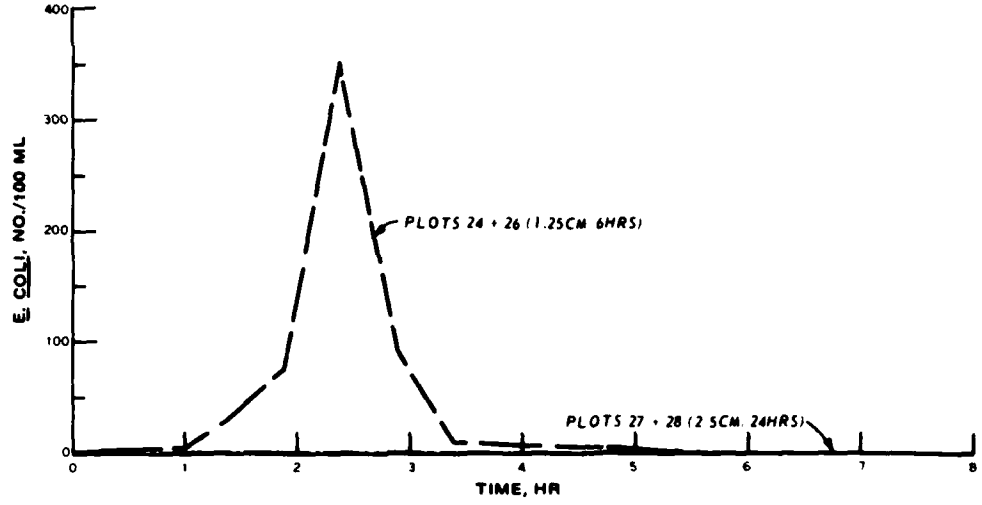
Plot 24 - Continuous flow, changed from intermittent flow 1 day before inoculation: 0.11 cm/hr, 24 hr/day

Plot 26 - Identical to Plot 24

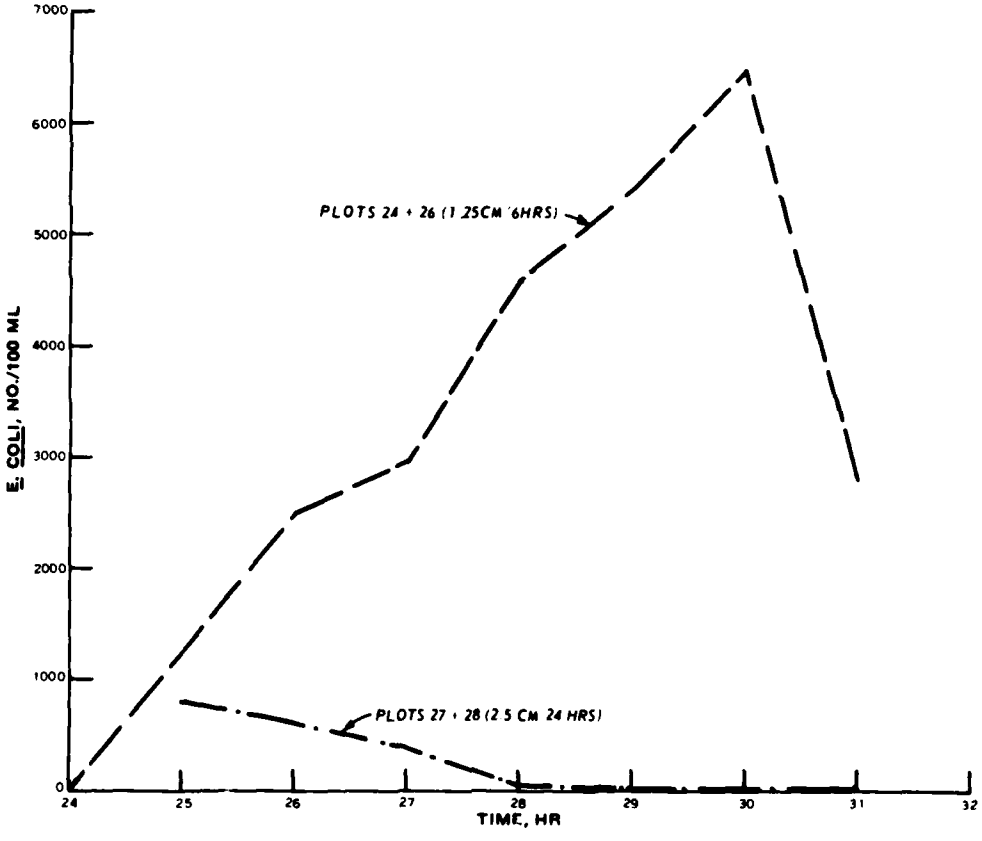
Plot 27 - Continuous flow, changed to twice the previous flow rate 1 day before inoculation: 0.21 cm/hr, 24 hr/day, 7 days/week

Plot 28 - Identical to Plot 27

Wastewater was continuously applied to all of the six plots, which were



a. Day 1



b. Day 2

Figure 8. Initial study of tracer E. coli numbers in runoff from Utica plots receiving wastewater intermittently (Plots 24 and 26) and continuously (Plots 27 and 28) (Note: plot inoculation at zero time)

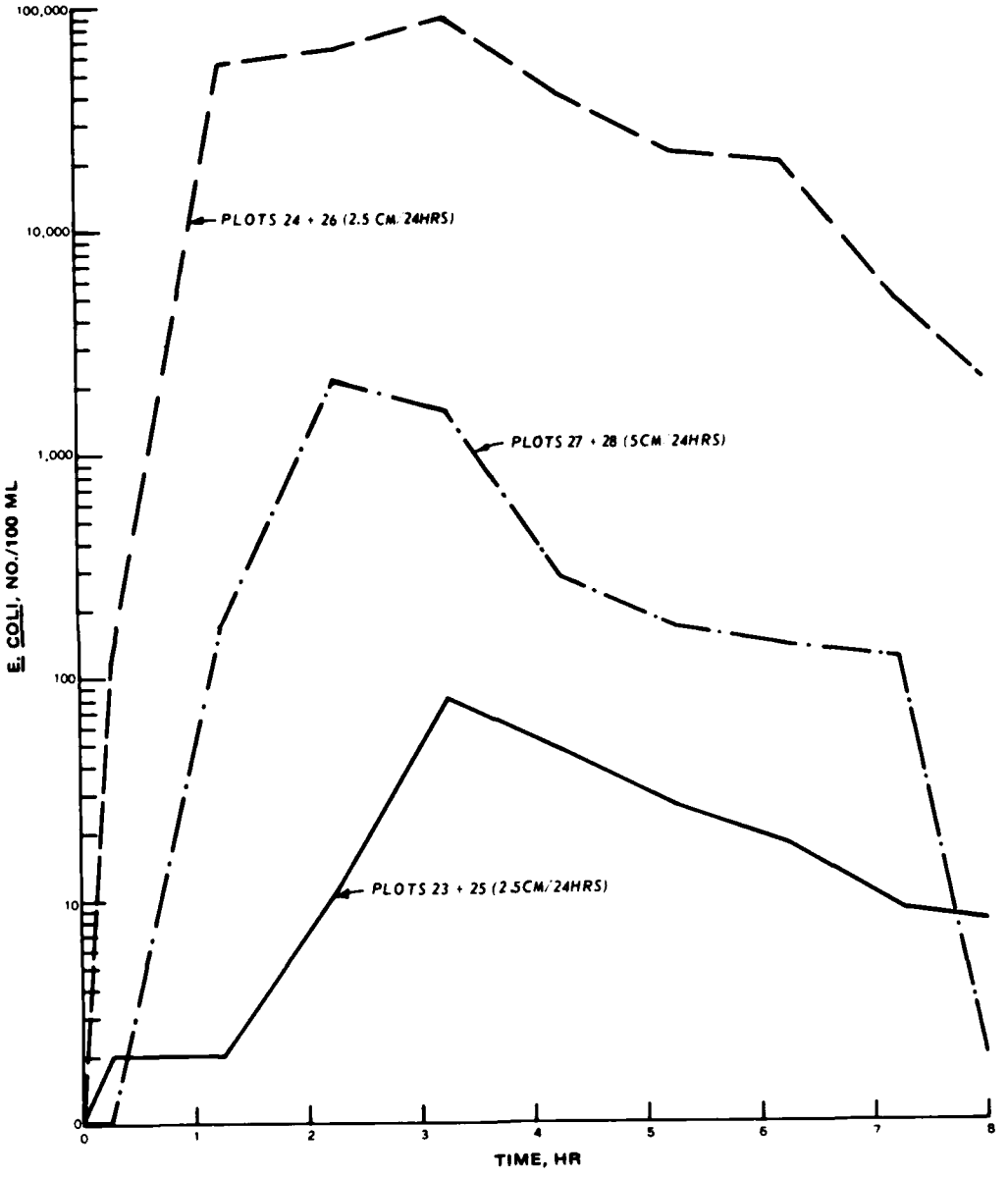


Figure 9. Second study of tracer E. coli numbers in runoff from Utica plots comparing different wastewater flow rates with flow durations (Note: plot inoculation at zero time)

at a 2 percent slope. Analysis of effluents from Plots 24, 26, 27, and 28 before the final test showed extremely low counts of the tracer E. coli added earlier.

69. The second tracer E. coli study attempted to clarify several assumptions acquired from the initial study. The main purpose of the various changes in flow rate and duration was to determine if the primary mechanism for fecal coliform removal on the plot was the flow rate or the physicochemical/biological conditions promoted by continuous wastewater application. Plots 23 and 25 were comparable to the previously monitored Plots 27 and 28 with the same flow rate and duration. Plots 24 and 26 served to test the influence of reduced flow rate on treatment efficiency since 1 day of acclimation was considered insufficient to significantly alter the physicochemical or biological nature of the system. Plots 27 and 28 served to test mainly the influence of physicochemical or biological features promoted by continuous wastewater application, since the increased flow rate was equivalent to that of Plots 24 and 26 in the initial study.

70. The results of the first study showed a peak of tracer E. coli in effluent samples from Plots 24 and 26 that were collected between 2 and 3 hr after inoculation (Figure 8a). The peak correlated well with earlier detention time studies on these plots using chloride ion as the tracer (see Table A2), which indicated that the peak represented bacteria not adsorbed on the plots. Since each inoculum was added to Plots 24 and 26 after effluent runoff occurred, the movement of the tracer E. coli was dependent on a constant runoff rate. No tracer bacteria were isolated from the first day samples from Plots 27 and 28.

71. On the second day of monitoring, the tracer E. coli on the intermittent-flow plots were detected in the first runoff sample (2 hr following renewed wastewater application) at 7 times the average number detected with the previous day's peak (Figure 8). Tracer bacterial numbers continued to climb rapidly for 6 hr (30 hr following test initiation) until an average peak of 6500 colonies per 100-ml sample was recorded (Figure 8). The count then declined with a declining runoff flow rate. Plots 27 and 28 showed no effluent peak. The peak on the

continuous-flow plots probably occurred during the night, aided by early morning rainfall, since only a declining curve was noted on the second day. Maximum numbers of tracer bacteria recovered from the continuous-flow plot effluents were about 1500 counts per 100 ml.

72. The second study, using three combinations of flow rate and duration on the six plots, showed broad plug flow peaks for all plots (see Figure 9). The peak for Plots 23 and 25, at a continuous application of 0.11 cm/hr, occurred at 3.25 hr. The initial peak for Plots 24 and 26, which had been recently switched to 0.11 cm/hr, also occurred after 3.25 hr; short circuiting down channels was evident by a rapid rise in tracer E. coli numbers within less than an hour after inoculation. The peak for Plots 27 and 28, which were eluted with twice the flow rate (0.21 cm/hr for 24 hr), showed the plug flow peak after 2.25 hr, although a significant decline in the broad peak did not occur until after 3.25 hr (see Figure 9).

73. The tracer E. coli concentrations in effluents from the plots during the second study are shown in Figure 9. The E. coli counts were much higher than those encountered during the first day of the earlier study. The reasons for the increased counts are not clear, since the inoculum used for the initial test was more than ten-times higher than that of the second run. Considering the extremely large numbers of tracer E. coli used as an inoculum, the eluted numbers are extremely inconsequential, with the greatest total cumulative effluent count for any plot being less than 0.01 percent of the respective inoculum number.

74. Two factors are evident from the two tracer E. coli studies, namely, that both the reduced wastewater flow rate (providing longer detention and greater soil contact) and the longer daily duration of wastewater application (modifying the physicochemical or biological conditions on the plots by continuous wastewater application) appear to improve the removal efficiency of fecal coliform bacteria. The thousandfold increase in tracer E. coli recovered from Plots 24 and 26, compared with Plots 23 and 25 at a comparable influent flow rate, seemed to be caused in part by short circuiting down channels on Plots 24 and 26. However, the almost hundredfold lower numbers encountered on Plots 27

and 28, which were run at twice the flow rate of Plots 24 and 26, strongly suggest that different removal mechanisms may also be involved.

75. Results suggesting that more efficient removal mechanisms were operative on Plots 27 and 28 include (a) the very low bacterial numbers encountered in Plot 27 and 28 effluents on the second day of the initial study, and (b) the rapid decline in tracer bacterial numbers on Plots 27 and 28 after 7 hr in the final study. The tenfold difference in numbers between Plots 23 and 25 versus Plots 27 and 28 appears to be a more realistic estimate of the impact of doubling the flow rate; each of these plots appeared to be comparable in detention time and in observed physical and biological states. The continuous-flow plots showed a conspicuous loss of grass and the development of a black-colored organic layer on the soil surface at the upslope end of each plot as a result of continuous flooding, as shown in Figure 10. The impact of these conditions on fecal coliform removal efficiency will be discussed in Part V.

Greenhouse Model Studies

76. Greenhouse horizontal soil lysimeters were used to duplicate and evaluate observed field processes at unseasonal times of the year. Summer temperatures and day length were employed in the greenhouse during winter months to stimulate the growth of microorganisms dominant during warm weather conditions. Three models were studied.

77. The first model contained 14-cm-thick sod collected from outside the Utica treatment plots. The model, prepared more than a year before this study, was considered to contain soil and grasses not recently contaminated by fecal material from warm-blooded animals. The bacterial isolates should have been natural inhabitants of the grassland environment.

78. The second model contained 14-cm-thick sod collected from down the length of a field plot at the Utica site. The sod had recently been contaminated by lagoon wastewater applied to the plot for 2 years prior to the time of collection. This model was allowed to equilibrate

a. Intermittent-flow plot

b. Continuous-flow plot

Figure 10. Views of intermittent- and continuous-flow plots showing variance in grass growth and development of a surface organic layer

for 2 months prior to the application of an E. coli strain. Therefore it was considered to be contaminated by a mixed flora of natural grassland and wastewater microorganisms, which persist or grow in a grassland environment.

79. The third model contained 14-cm-thick sod collected from near the feeding area of a cattle pasture at Utica, Miss. Fresh cattle manure was added twice each week to the model. Since manure was only applied to the upper third of the model, effluent monitoring was intended to determine whether or not fecal coliform bacteria derived from manure would be readily leached downslope through soils similar to those found at the overland flow system at Utica. Overland flow systems are often accessible to numerous small warm-blooded animals, whereas at the Utica site periodic invasion of the plots by stray cattle had occurred. An added feature of the greenhouse models was the control of fecal contamination derived from extraneous warm-blooded animals and most insects, which are considered as sources for fecal coliforms in runoff from uncontaminated land surfaces.[50]

Model 1

80. Model 1 (untreated Utica sod) received deionized water containing a morphologically distinct strain of E. coli, which formed a crusty blue colony with a pinkish center on M-FC medium. This bacterium was originally isolated from effluent runoff from the Utica overland flow treatment plots, and other bacterial isolates with this same morphology consistently keyed to E. coli (excellent to very good identification) with API biochemical strips. Previous runs of the model prior to treatment with the E. coli showed no similar colonies on M-FC membrane filter plates. Effluent isolates were periodically checked with the API strips for verification. A 1- to 5-ml aliquot of the distinctive E. coli was mixed in a barrel of deionized water, and this influent was applied to Model 1 at a rate of 1.25 cm in 6 hr, 5 days/week.

81. The data obtained from Model 1 tests are depicted in Figure 11; each daily mass removal efficiency is given above each daily set of influent and effluent fecal coliform counts per 100-ml sample. Corresponding data are shown in Table B2, including several multiple analyses

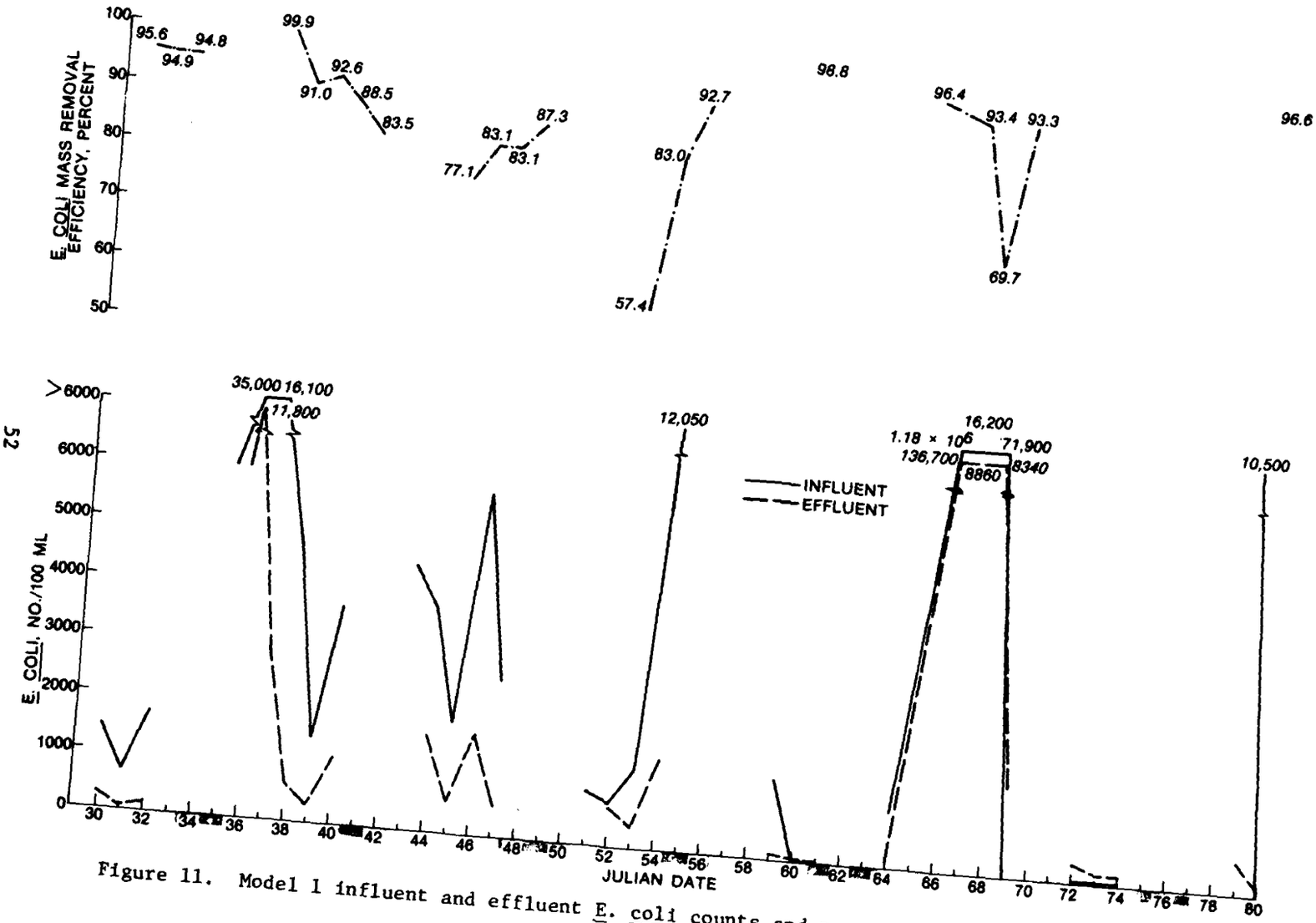


Figure 11. Model 1 influent and effluent E. coli counts and mass removal efficiencies

of given samples after storage periods of 2-3 days at 4°C. Only the initial set of data was incorporated into Figure 11. The trends indicate fecal coliform mass removal efficiencies generally in excess of 90 percent with an average of 93.4 percent. Removal efficiency based on influent and effluent concentration was 87.5 percent. Larger influent doses generally gave better removal efficiencies, but they also caused excessive numbers of E. coli in effluent samples. Excluding the very high influent and effluent counts obtained on Julian date 66, the average mass removal efficiency decreased to 89.7 percent, but average E. coli counts in effluent samples also declined from 6370 to 1875 per 100-ml sample, respectively.

82. The initial effluent runoff generally contained E. coli counts comparable to later or composite samples (Table B2). This indicated that sorption of discrete bacterial cells to the soil occurs rather uniformly over the treatment period, probably only when cells are in intimate contact with the soil surface. There appeared to be a latent effect of about a day following a given high-dose treatment. Die-off of this E. coli strain from the model surface was rapid, and a level of less than 200 counts per 100-ml sample was usually reached after about 3 days with no inoculation (Figure 11; Table B2).

Model 2, first study

83. Model 2 (Utica field plot sod) was initially treated with high numbers of a tracer strain of E. coli, namely, one with genetic resistance to the antibiotic streptomycin. As such, the influent E. coli could be separated from soil-borne fecal coliform bacteria in effluent samples. The tracer bacterium was added to 70 ℓ of deionized water, which was applied to the upslope margin of the model at a rate of 0.21 cm/hr/6 hr each day, 5 days/week.

84. The data for the 2 months of monitoring with the tracer E. coli strain are given in Table 1. The average influent concentration of almost 42×10^6 E. coli per 100-ml sample is about a thousandfold higher than the high values encountered in influent wastewater at the Utica overland flow plots. Despite these very high numbers, effluent E. coli counts were reduced by over 87 percent with mass removal efficiency

approaching 92 percent (Table 1). Effluent runoff quality was far above the currently accepted level of 200 counts per 100-ml sample.

85. Influent values were kept fairly constant during this monitoring program. Similarly, effluent concentrations showed minor variation, with average numbers approximating 5×10^6 cells per 100-ml sample. No difference was noted between early (Tuesday) and late (Thursday) weekly effluent reductions with removals of 86.2 and 88.1 percent, respectively. Average runoff volumes were comparable on these two sampling days. Die-off of this E. coli strain to low levels appeared to be rapid since rather uniform removal efficiencies were encountered during the course of experimentation. The majority of the daily effluent counts appeared to originate from the influent loading for the same day.

Model 2, second study

86. The second study conducted on Model 2 involved the application of Utica algal lagoon wastewater as an influent. No additional bacteria were amended to the influent. Normal variations in countable fecal coliforms in the influent wastewater were monitored with each daily effluent sample analysis. The major purpose of this study was to assess what effect changing the influent wastewater flow rate might have on fecal coliform removal efficiency under more controlled greenhouse conditions. The flow rates and the order of evaluation included 0.21 cm/hr for 6 hr, 0.07 cm/hr for 18 hr, 0.14 cm/hr for 18 hr, and 0.21 cm/hr for 18 hr. All of these flow rate conditions were maintained for 5 days each week, Monday through Friday.

87. Data for the two initial flow conditions, namely, 0.21 cm/hr for 6 hr and 0.07 cm/hr for 18 hr, are given in Table 2. Data collected from the remaining two flow rate conditions were discarded because of surface flow interferences caused by earthworm mounding under the more continuous water application condition. This culminated in a serious reversion to a subsurface flow pattern by the end of the third flow rate monitoring; surface flow variation was not noted during the first two flow rate adjustments.

88. The data in Table 2 show two interesting trends: (a) the fecal coliform effluent counts during the higher, more intermittent

wastewater application were nearly a hundredfold greater than counts obtained at the lower, more continuous rate, e.g., 14,000 and 180 counts per 100-ml sample, respectively; and (b) the number at the higher flow rate was almost tenfold greater than contemporary influent fecal coliform enumerations. These trends persisted for a day into the second flow rate monitoring; the model adjusted to the new flow conditions at that time and then elicited an average 73 percent fecal coliform treatment efficiency. Despite the greatly increased efficiency at the lower flow rate, very low influent counts usually corresponded with slightly higher effluent counts. The highest daily removal efficiencies usually occurred when high influent counts were observed.

89. The increased numbers of fecal coliform bacteria enumerated in runoff from the model, compared with influent counts, were initially assumed to be due to failure of many viable wastewater bacteria to grow on the highly selective M-FC medium of the membrane filter test. Certain stress factors in the lagoon wastewater were thought to induce reversible cellular damage to many of the fecal coliform bacteria and that their recovery occurred as a result of overland flow across the sod. High pH was suspected as a stress factor since a high pH was observed in the lagoon wastewater during the summer. However, periodic monitoring of influent wastewater pH, including measurements after different storage intervals, gave an average value of 7.05 (range: 6.65 to 7.4), compared with an effluent value of 6.75 (range: 6.35 to 7.1). Elevated temperature or rapid temperature fluctuations could also have induced stress,[13,24] since the wastewater was obtained from the lagoon during the cold winter months and stored during the week of application in the greenhouse. Air temperatures in the greenhouse, averaging 24.5°C (range: 20 to 33°C), were usually not excessive. Overland flow water temperatures were also not excessive (Table A4). An assessment of whether or not wastewater stress factors were important for the observed fecal coliform response was accomplished by a two-temperature M-FC membrane filter test modification. The test, involving initial incubation at 35°C for 4-5 hr followed by 18-hr incubation at 44.5°C,[93] was compared with the standard test for several influent samples. The small

differences noted were not sufficient to elicit the fecal coliform increases observed in the effluents.

90. The unique occurrence of increasing fecal coliform numbers in overland runoff from the greenhouse models was never observed when deionized water containing different E. coli inocula was being applied to this model. The similarity of this trend with summer field observations strongly infers that the wastewater properties must be inducing the growth of fecal coliform bacteria on the soil surface of the overland flow test plots. Nutrient data for Model 2 effluents, collected during the application of deionized water (Table A3), show that total and soluble nitrogen and phosphorus species leached from the model were within the range of values monitored in the lagoon wastewater (Table A1). Therefore, other factors seem to be involved besides fecal coliform growth stimulation by nutrients in the wastewater. Suspended organic solids in the wastewater may have served as niches for the survival and/or growth of fecal coliform bacteria. The impact of suspended solids in the wastewater on the observed greenhouse and field plot results will be discussed in detail in Part V.

91. The highest fecal coliform numbers in effluents from the intermittent-flow treatment were counted in the initial runoff, which followed the weekend of inactivity (Table 2). The numbers then declined during the course of the 5 days of wastewater application. This decreasing trend was also observed for the stored lagoon wastewater, which was replenished with fresh wastewater every Monday. However, the greatly accentuated effluent numbers clearly indicate that some growth of fecal coliform bacteria was occurring on the model and that this growth was greatly accentuated by intermittent-flow conditions.

Model 3

92. The third greenhouse model was filled with grass sod from an actively grazed cattle pasture near the Utica overland flow field site. Purposes of the model included the following:

a. Evaluating if runoff from Utica pastureland, or adjacent arable land receiving uncomposted manure or wastewater sludges, might exceed present point-source criteria for fecal coliform bacteria.

b. Determining the bacterial sorptive capacity of Utica soils that were continuously subjected to nonhuman contamination but not to continuous application of domestic wastewaters.

c. Studying the die-off characteristics of fecal coliform bacteria originating from cattle manure.

d. Determining if pasture runoff is an important source for bacteria that interfere with the membrane filter test enumerations of E. coli.

93. The quantity of cattle manure added to Model 3 was based on the fecal loading expected for an average Mississippi pasture.[48] The recommended live weight stock loading is about 1000 kg/ha,* with an effective annual manure coverage of 20 percent.[48] The dry weight manure loading for pasturage of 1000-kg live weight cattle per day was calculated to be 1.86 kg/ha/day, based on two sources of information.[48,103] This is equivalent to 6.45 g dry weight manure per day on the greenhouse model. A lower cattle manure loading of approximately 3 g was added to the upper third of the model to avoid unrealistic overtreatment caused by increased coverage. A freeze-dried manure was added initially but it gave very low fecal coliform counts. Therefore, a fresh manure slurry, consisting of 30 g wet weight manure in 500 ml deionized water, was added every Tuesday and Thursday. This loading was equivalent to 6 g dry weight manure per week.

94. The manure inocula were leached across the model surface by applying deionized water to the model at an application rate of 0.21 cm/hr for 6 hr/day, 5 days/week. The mass loading of fecal coliform bacteria was determined by measuring counts in sample aliquots of the manure slurry for each day of application. Surface runoff was monitored for fecal coliform bacteria every Tuesday and Thursday; effluent volumes were recorded to determine mass removal efficiency. Data from this study are given in Table 3.

95. The average mass loading of fecal coliform bacteria approached 15×10^8 cells per application (Table 3). Biochemical

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* Personal communication, Mr. Hunter George, County Agent, Warren County, Miss., June 1979.

testing showed that a very high percentage of the positive colonies on M-FC membrane filter plates were E. coli. The initial runoff usually showed the highest fecal coliform numbers, as would be expected. However, when the fecal coliform inoculum number showed tenfold reductions, this trend generally reversed. Although the highest treatment efficiency resulted from the highest mass loadings with fecal coliforms, the respective effluent concentrations of fecal coliforms also usually increased; average counts exceeded 500 per 100-ml sample. The mass removal efficiency exceeded 99.5 percent during most of the monitoring program. Most of the effluent isolates also keyed to E. coli with biochemical testing although other bacteria giving a positive test were occasionally isolated. These data indicate that fecal coliform bacteria in land runoff from pastureland could result in counts consistently in excess of the present acceptable level of 200 per 100-ml sample.

Enumeration Problems with the Standard Membrane Filter Test

96. Interference problems, caused by the growth of large numbers of natural soil bacteria on the standard M-FC membrane filter test plates, have been mentioned in the preceding sections. This interference resulted in a number of different phenomena:

a. Suppression of the growth of true fecal coliform bacteria, namely, different E. coli strains.

b. Prevention of proper blue color development of fecal coliform bacterial colonies on the plates by nonfecal isolates.

c. Synergisms between a true fecal coliform bacterium (e.g., E. coli) and a species indigenous to the land treatment system, resulting in false positive counts. (False positive bacteria cannot ferment the lactose in the M-FC medium in the absence of the growth of other microorganisms.)

d. The growth of fecal coliform bacteria (e.g., Klebsiella and Enterobacter species) that are not indicative of recent fecal contamination, and thus would not be representative of actual disease potential.

The foregoing synopsis strongly suggests that a selective test for

E. coli, rather than for fecal coliform bacteria in general, is desirable for evaluating the pollution potential of algal lagoon wastewaters and overland runoff.

97. The identification of bacteria isolated on M-FC medium-saturated membrane filters, following 24-hr incubation at 44.5°C, was accomplished with API-20E biochemical test strips. Representative samplings of the nearly 150 bacterial isolates cultured from M-FC plates are shown in Tables 4, 5, B3, and B4, with their positive or negative reaction to each of 20 biochemical tests. Table 4 depicts isolates, other than E. coli, that gave a positive blue colony color on the M-FC test plates at low total counts, e.g., usually less than 100 per plate. All of these isolates are capable of breaking down lactose (ONPG +), which is the main carbon source of the M-FC medium. All of the identified isolates were fecal coliforms, namely Klebsiella pneumoniae, Enterobacter species, and two questionable (citrate + and indole -) E. coli.[83,84] Table 5 shows isolates that gave a positive blue colony color only when plate numbers exceeded the limit of 200 total counts per plate recommended by Millipore Corporation[104] and the 120 count limit recommended by Gelman Instrument Company.* The primary false positive isolate from the Utica field plot effluents was Acinetobacter calcoaceticus var. anitratus. Many of the Klebsiella isolates could also be included with this table, as they frequently gave a deep blue colony color only at high plate counts. The diverse morphological and biochemical variability of the E. coli and K. pneumoniae isolates encountered are apparent from the data in Tables B3 and B4, respectively.

98. A few common morphological observations were made among the various fecal coliform and false positive isolates. For example, about 90 percent of the E. coli formed crusty, nonmucoid colonies, and about three quarters of these isolates displayed a characteristic blue-colored colony with a pinkish central area (Figure 12). A blue colony with a pink center very seldom keyed out as anything but a definite E. coli.

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* Personal communication, Mr. W. G. Presswood, Chief Microbiologist, Gelman Instrument Co., Ann Arbor, Mich., May 1979.

The only exception was culture 40 (Table 4); a negative indole test prevented an excellent identification of this isolate as an E. coli. Colonies of E. coli lacking the characteristic pink-centered blue color are shown in Figure 13. Most of the Klebsiella and false positive isolates possessed mucoid colonies (Figures 12, 14, 15, and 16; Tables 4, 5, and B4). The most common Enterobacter isolate was E. cloacae, which formed a blue colony with a diffused (fuzzy) margin at low plate numbers, as shown in Figure 16. Thus, colony morphology should aid in separating E. coli from other fecal coliform or false positive bacteria, although one must be aware that notable exceptions exist, for example, the watery to mucoid E. coli colonies depicted in Figures 13 and 17.

99. The K. pneumoniae colonies were often light blue (at low plate numbers) upon immediate inspection of the M-FC test plates. However, their colonies usually lost the blue color rapidly, often within 30 min after exposure to the air. Unfortunately, this color change by K. pneumoniae generally reversed the color of the entire medium from blue to grey or pink (Figure 15b), and also resulted in a loss of blue color of any accompanying E. coli colonies. The A. anitratus also commonly formed blue colonies on M-FC test plates but only in the presence of E. coli colonies (Figure 18). When the E. coli colonies were at low numbers (e.g., less than the recommended count) and the A. anitratus colonies were at numbers greater than 300 per plate, the E. coli colonies would develop a pink or off-blue color whereas those of A. anitratus would show a deep blue color (Figure 18). This is one of several examples noting microbial synergism, where more than one microbe is required to ferment the lactose in the selective M-FC medium at an elevated temperature and to produce the required acidic pH to turn the aniline blue dye in the M-FC medium to the proper blue color. According to Bagley and Seidler,[82] most colonies will not turn blue on the M-FC medium until the pH is less than 5.3.

100. Another possible synergism was between culture 118, identified as a highly questionable Enterobacter species, and a blue pin colony (Figure 19). The culture 118 isolate could break down lactose whereas the blue pin colony could not; the latter was blue probably only at numbers

E. coli, rather than for fecal coliform bacteria in general, is desirable for evaluating the pollution potential of algal lagoon wastewaters and overland runoff.

97. The identification of bacteria isolated on M-FC medium-saturated membrane filters, following 24-hr incubation at 44.5°C, was accomplished with API-20E biochemical test strips. Representative samplings of the nearly 150 bacterial isolates cultured from M-FC plates are shown in Tables 4, 5, B3, and B4, with their positive or negative reaction to each of 20 biochemical tests. Table 4 depicts isolates, other than E. coli, that gave a positive blue colony color on the M-FC test plates at low total counts, e.g., usually less than 100 per plate. All of these isolates are capable of breaking down lactose (ONPG +), which is the main carbon source of the M-FC medium. All of the identified isolates were fecal coliforms, namely Klebsiella pneumoniae, Enterobacter species, and two questionable (citrate + and indole -) E. coli.[83,84] Table 5 shows isolates that gave a positive blue colony color only when plate numbers exceeded the limit of 200 total counts per plate recommended by Millipore Corporation[104] and the 120 count limit recommended by Gelman Instrument Company.* The primary false positive isolate from the Utica field plot effluents was Acinetobacter calcoaceticus var. anitratus. Many of the Klebsiella isolates could also be included with this table, as they frequently gave a deep blue colony color only at high plate counts. The diverse morphological and biochemical variability of the E. coli and K. pneumoniae isolates encountered are apparent from the data in Tables B3 and B4, respectively.

98. A few common morphological observations were made among the various fecal coliform and false positive isolates. For example, about 90 percent of the E. coli formed crusty, nonmucoid colonies, and about three quarters of these isolates displayed a characteristic blue-colored colony with a pinkish central area (Figure 12). A blue colony with a pink center very seldom keyed out as anything but a definite E. coli.

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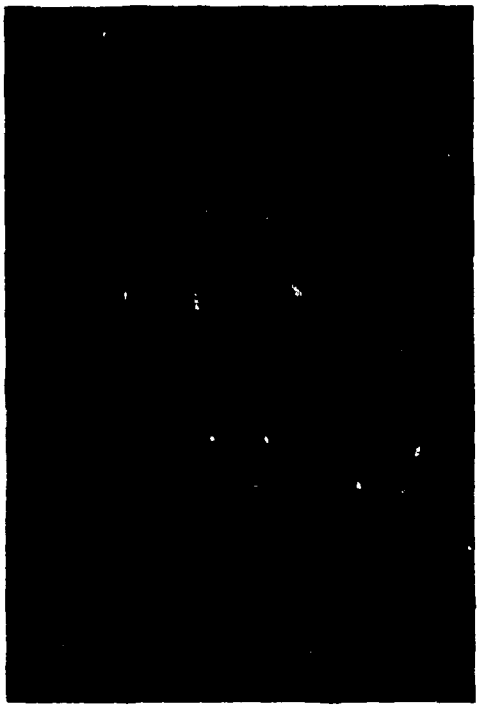


Figure 12. Pink-centered E. coli with K. pneumoniae colonies on M-FC test plate

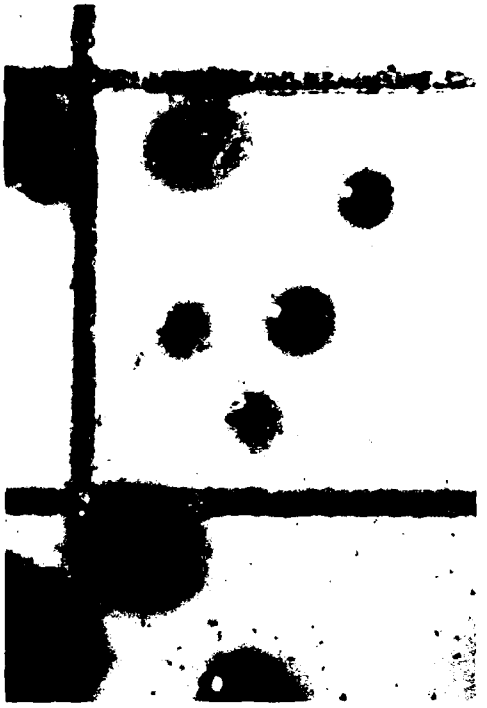


Figure 13. Crusty and mucoid E. coli colonies lacking pinkish centers on M-FC test plate

Figure 14. Colonies of K. pneumoniae causing positive interference with E. coli (right plate). A fivefold dilution causing loss of blue color in the K. pneumoniae colonies (left plate)

a. Plates immediately following incubation

b. Plates after 20 hr storage at room temperature

Figure 15. Colonies of K. pneumoniae and E. coli on M-FC test plates showing false negative and positive interferences (Note: blue color changes are observed with fivefold dilutions and after plate storage; the influent plate (upper left) contains only E. coli)

Figure 16. Colonies of E. cloacae (fuzzy margin) and pink-centered E. coli on an M-FC test plate

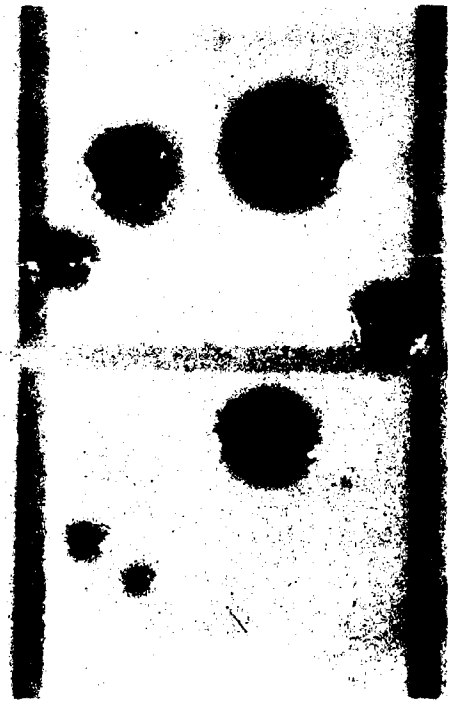


Figure 17. Mucoid and pin-sized E. coli colonies on M-FC test plate

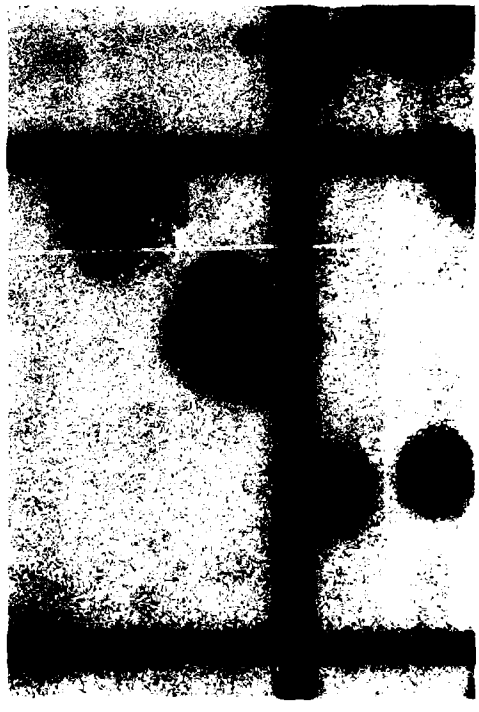
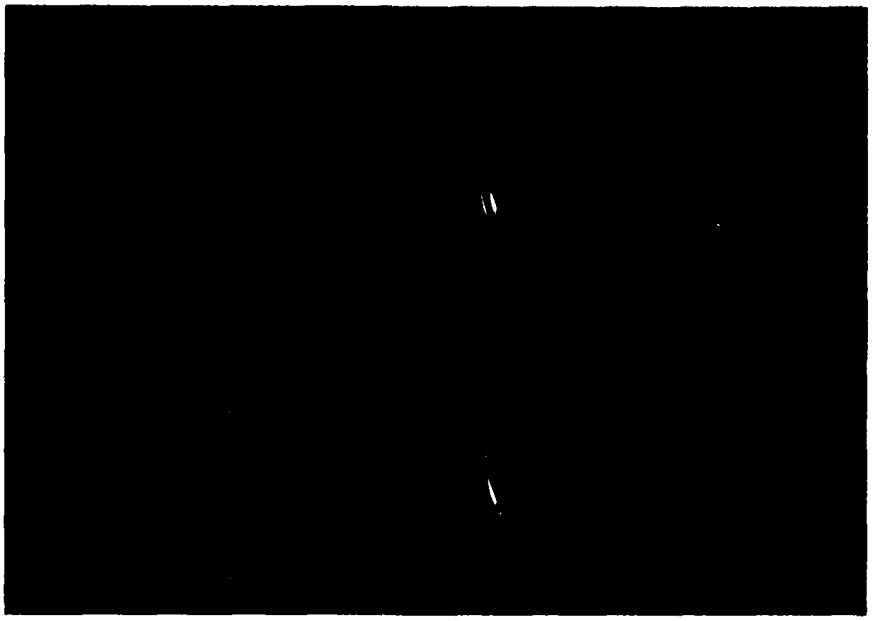


Figure 18. Blue A. anitratus colony causing blue color loss in an adjacent E. coli colony



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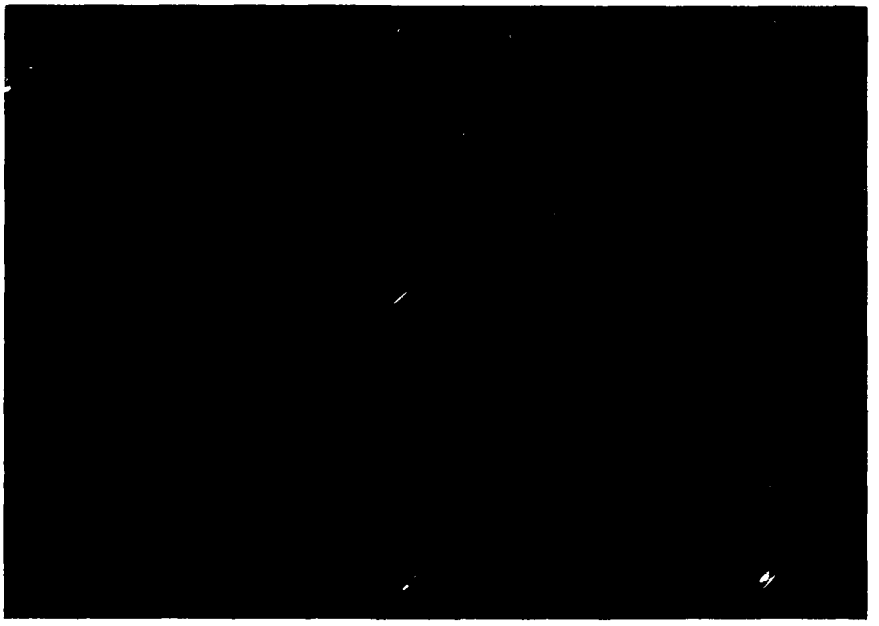

b. Plates after 20 hr storage at room temperature

Figure 19. Culture 118 (large flat) colonies with false positive blue pin colonies showing a possible synergism (Note: the colonies remaining blue on the M-FC test plates after 20 hr storage are E. coli)

greater than about 200 per plate (even higher numbers were required for a blue color in the absence of culture 118). The unusual number of positive biochemical tests for isolate 118 (Table 4) and the contamination of two separate cultures by the pin colony bacterium indicated that both microbes grew naturally in a very close association and probably acted synergistically. Loss of isolate 118, which was contaminated by the pin colony, precluded any valid follow-up testing. The synergism probably did not include an E. coli since incubation of an E. coli with the pin colony for different time periods and at various concentrations failed to recreate a colony with the characteristics of culture 118. Following overnight storage at room temperature, culture 118 lost the blue color, whereas the associated E. coli colonies remained blue. All blue colonies remaining on the effluent plates shown in Figure 19b keyed out as E. coli, using the API biochemical test strips.

Fecal Coliform Response at High and Low Temperatures

101. Previous field and greenhouse studies indicated that fecal coliform bacteria might be growing on overland flow systems receiving intermittently applied lagoon wastewater. To further test this hypothesis as well as to evaluate the interaction of high versus low temperature on the growth or die-off characteristics of fecal coliforms, a controlled-temperature test was performed in a growth chamber. Six intact grass sod squares were removed from adjacent areas of an overland flow field test plot that received wastewater intermittently. The sod was field saturated with lagoon wastewater at the time of collection. Each square of sod, measuring 31 by 31 by 8 cm, was tightly packed into a polypropylene pan and each replicate set of three sod-filled pans was then placed in growth chambers set at 10° and 37°C, respectively. Lighting was set to simulate summer diurnal conditions. Following a 6-day incubation period, deionized water was added to each pan to cover the soil surface by approximately 1 cm. The pans were then incubated for an additional 3 hr at each respective temperature. Surface water samples were then carefully removed from each pan with a syringe and enumerated for fecal

coliform bacteria with the standard M-FC membrane filter test.

102. The test results are given in Table 6. These data showed trends that were very similar to the field observations. At elevated temperatures similar to summer field conditions at the overland flow system (i.e., 37°C), the numbers of interfering fecal coliform and false positive bacteria became very numerous on the soil-grass system, probably as a result of in situ growth. Cool temperatures (i.e. 10°C) did not promote the growth or persistence of interfering bacteria, namely, bacterial colonies other than E. coli. The presence of numerous interfering colonies on the fecal coliform test plates precludes the ability to accurately count the E. coli colonies; these colonies can cause both positive and negative counting problems even for experienced technicians. The findings showed that the E. coli numbers were not greatly changed by the experimental temperature variance since relatively uniform counts were obtained at both the high and low temperatures. Although the results did not substantiate the capability of E. coli to proliferate on an overland flow system, they certainly confirmed the persistence of E. coli for at least 6 days both at 10° and at 37°C.

Comparative Testing of Alternate Membrane Filter Procedures

103. Problems inherent in enumerating fecal coliform bacteria in overland runoff waters prompted the evaluation of other tests and a modification to the standard M-FC test. Two variations of the M-TEC test were evaluated, namely, a two-step procedure involving filter transfer to a urease solution to separate E. coli (urease -) from Klebsiella[97] and a single-step procedure using indoxyl-β-D-glucoside in the primary medium.[98] Media compositions for these tests are given in Tables A5 and A6. An M-FC test modification, involving preincubation at 35°C,[93] was also evaluated.

104. The standard M-FC test[5,7] was compared with the two M-TEC tests. The two-step M-TEC test was found to be superior to the single-step M-TEC modification by virtue of better colony color differentiation and higher verified counts (based on API strip biochemical testing).

Evaluation was based on the analysis of only seven samples, including two stock E. coli cultures. The computed means for the standard M-FC and two-step M-TEC test were 427 and 541, respectively, with the M-TEC counts being consistently slightly higher. Statistical analysis, using the F-test (with and without log transform to stabilize the variances), showed that the differences between the two tests were significant ($P < 0.05$). Despite this finding, colony color differentiation was generally poorer with the M-TEC test. Further comparisons, using land runoff samples, should be performed in order to adequately evaluate the M-TEC test in natural environments.

105. The modified M-FC test was also compared with the standard M-FC test. Statistical interpretation of results was broken into 15 field samples and four greenhouse model samples. The field data showed increased counts for the modified M-FC procedure; the means for the standard and modified tests were 461 and 565, respectively. The F-test showed significance at the $P < 0.05$ level with and without log transform. However, the greenhouse data, based on only a few samples, were not found to be significant. Since all test results tended to fluctuate in regular patterns, different kinds of stress were apparently operative at different times of the year. The differences between the standard and modified tests were not great enough to unconditionally recommend the modified M-FC test for evaluating land runoff. This and other modified testing procedures need further evaluation. Such intensified testing was beyond the scope of this study.

Effect of Sample Storage on M-FC Test Results

106. The collection and testing of field samples on the same day was often difficult because of the great distance between the overland flow field plots and the analytical laboratory. Therefore, comparative fecal coliform analyses of collected samples were performed following refrigerator storage of the samples at 4°C for less than 4 hr and for approximately 24 hr, respectively. A similar study was performed on samples collected from a greenhouse model (Table B2). All fecal coliform

determinations were made with the standard M-FC membrane filter test.

107. The data collected from Utica field plot samples showed that a small, generally consistent decrease in fecal coliform counts followed the overnight storage of samples at 4°C. An F-test analysis of 14 comparisons showed that the difference between the means from the two incubation periods was highly significant ($P < 0.01$) with and without log transform. Mean fecal coliform counts for short (4 hr) and long (24 hr) refrigeration periods prior to testing were 4365 and 2680, respectively.

108. Greenhouse model effluent samples, collected during the application of a morphologically distinct strain of E. coli in deionized water as an influent, showed a small decrease in fecal coliform counts following the overnight refrigeration of six samples. The decrease to 83 percent of initial test results, after an additional 24-hr storage of the same samples at 4°C, was not found to be significant ($P < 0.05$, using the F-test). However the general trend was a small decline in numbers, as indicated by the data in Table B2.

109. The trend for declining fecal coliform counts was occasionally drastically reversed. For example, one set of samples from Plots 24, 26, 27, and 28 at Utica (3 October 1979) showed very large increases in counts following overnight sample storage at 4°C. Fecal coliform enumerations were as follows:

a. Same day testing - Wastewater: 873 counts/100 ml;
   Plots 24 and 26: 6700 and 2200 counts/100 ml;
   Plots 27 and 28: 160 and 436 counts/100 ml.

b. Second day testing - Wastewater: not run;
   Plots 24 and 26: 95,700 and 48,000 counts/100 ml;
   Plots 27 and 28: 25,700 and 6,300 counts/100 ml.

The reason for these dramatic increases is not clear. Background bacerial interference on the plates did not appear to be involved since total colonies were less than 50 per plate at the lower dilutions, and two tenfold dilutions gave comparable numbers. Recovery of stressed bacteria in the samples during overnight storage was possible, although the source for any such stress is unknown since the wastewater sample was not run on the second day. Growth of the fecal coliform bacteria at 4°C during a 24-hr period was also possible. Despite the cause(s) for

the great variances, the reason why this particular sample set promoted increasing enumerations remains elusive.

110. An answer to the question of whether overnight storage gives a better or poorer estimate of actual viable fecal coliforms in a sample remains for further research. However, occasionally serious enumeration problems can exist from the overnight storage of samples from overland flow systems.

PART V: DISCUSSION

Fecal Coliform Enumeration Problems

111. Many fecal coliform enumeration problems were prevalent during the conduct of this study. Similar problems were experienced when the Utica lagoon wastewater, overland runoff from the Utica field test plots, and effluents from three greenhouse overland flow models were monitored. The frequently large fluctuations in overland flow field plot influent and effluent fecal coliform counts, using the standard M-FC membrane filter test,[5,7] were also experienced in the greenhouse when lagoon wastewater was added as an influent to an overland flow model. These fluctuations appeared to be caused not by a single phenomenon but by a combination of factors.

Seasonal fluctuations

112. Low fecal coliform counts in overland flow plot influents during the early spring and summer months were thought to be promoted by two entirely separate major events. Heavy spring rainfall, and a slow overturn of more recently contaminated surface water with more aged bottom lagoon wastewater during periods of extended cold, could have reduced viable numbers of fecal coliform bacteria in the plot influents during the early springtime. Low summer counts appeared to be promoted by several stress factors in the lagoon water; these would have decreased the ability of fecal coliforms to develop colonies on the highly biochemically selective M-FC medium, especially at the elevated incubation temperature of 44.5°C.[89,105,106] A literature summary by Skerry and Parker[107] concerning treatment mechanisms for algal lagoons indicates the complexity of interactions responsible for fecal bacterial reductions. Several factors mentioned include algal toxicity, high pH, and an exhaustion of the food supply. Temperature studies show mixed findings,[24,26,28,30] although rapid short-term growth followed by a more rapid die-off is generally observed at elevated temperatures.[24,30] Low levels of carbon dioxide may also prevent growth of *E. coli*.[44] All of these factors should be more prevalent during the warm summer months in

the Utica sewage lagoon. Although the very low influent counts observed from May to July during two years of field monitoring corresponded closely with elevated pH, a combination of factors was undoubtedly involved. For example, the combination of high pH and ammonia released from decaying lagoon algae could provide toxic conditions for coliform bacteria.[101] Multiple stress factors appear to be most damaging to fecal coliform bacteria (i.e., E. coli), which lack the ability to adjust well to changing environmental conditions.[42]

113. Late spring and summer field conditions produced an interesting phenomenon at the overland flow field plots, namely, much higher fecal coliform counts in the effluents than those in the respective influent (lagoon wastewater) samples (see Figure 7). Despite the extremely low counts for many summer influent samples, the corresponding effluents occasionally gave very high counts: in excess of 10,000 per 100-ml sample. One possible cause was that the influent counts may not have been indicative of the viable fecal coliform population, due to environmental stress factors.[89] A proven test for overcoming many forms of environmental stress involves a 2- to 4-hr preincubation of M-FC filter plates at 35°C prior to an 18-hr incubation at the recommended elevated temperature.[93,108] Both the two-temperature and the standard M-FC tests were conducted on 14 field plot and lagoon wastewater samples collected during the summer. Although this test provided a significant increase in fecal coliform plate counts, the almost twofold higher numbers encountered could not explain the much larger differences that were observed. Since other tests[90-92,94] recommended to overcome stress were not used, the possibility that stressed fecal coliform bacteria might be recovering during residency on the overland flow plots cannot be validly substantiated. The point is that stress did play a significant role, and the presence of stressed indicator bacteria in wastewaters applied to the land should be assumed until several modified testing procedures have been performed to prove otherwise, as recommended by the EPA.[7]

114. The sporadically very high fecal coliform counts in runoff waters from the Utica field plots during summer months (see Figure 6) and from a greenhouse model during lagoon wastewater application (see Table 2)

were generally thought to be the result of bacterial growth on the overland flow system soils. Such growth seemed to be favored by intermittent-flow conditions, and the highest numbers were encountered following a 1- to 2-day lag in overland flow treatment. This apparently short-term growth was followed by a rapid decline after a few days and was facilitated by moderately high maximum soil temperatures, i.e., 25° to 35°C. An interesting point is that strong indications of growth, including effluent counts that exceed influent counts, were rarely observed when deionized water containing E. coli was added to the greenhouse models. Certain constituents in the wastewater were most probably involved in inducing this fecal coliform bacterial proliferation.

115. Considering all factors previously discussed in this paper, it seems most logical that the fecal coliform bacteria in the lagoon wastewater were primarily associated with the suspended organic solids. Bacterial survival was probably facilitated by the more uniform nutrient-enriched environment of the organic particles, which also could provide them limited protection from natural predators. Similar accentuated survival or growth has been noted in wastewater either when suspended solids levels were high[30] or following the addition of clay with a high sorption potential.[25] The frequently enormous increases of indicator bacteria in storm runoff from contaminated land surfaces[49,50,52,109] also document the common association of viable fecal bacteria with particulate matter. Low-density suspended organic solids could readily be transported the length of the treatment slope even at the low application flow rates. Following entrapment of these solids on the treatment system, extended residency on the nutrient-rich soil surface could allow for growth on the soil adjacent to particles harboring viable fecal coliform bacteria. Growth on the soil could not readily occur during continuous-flow conditions as intimate soil contact occurs most readily after drainage and soil dewatering. Fecal coliform proliferation on the intermittent-flow plots during nonflow conditions could lead to increased numbers in runoff waters after continued wastewater application.

116. The fall and early winter months were generally found to be most favorable for high numbers of fecal coliform bacteria in influents

and effluents from the field overland flow plots, based on M-FC test counts (see Figures 6 and 7). This trend was observed for the continuous and the intermittent wastewater applications. A combination of factors was probably involved: cooler seasonal temperatures, which could allow for longer survival of the fecal coliforms;[24,30] the abundance of organic detritus in the lagoon, which culminated from the decline of summer algal blooms;[30] and low fall rainfall during the monitoring program, which would preclude a dilution effect.

117. The usually high but sporadic early winter fecal coliform counts in influent and effluent samples from Utica were found to steadily decline during the late winter, based on limited data (see Figure 6 and Table B1). Effluents from the field plots showed the lowest average fecal coliform counts during February (see Figure 7). The coliform counts were consistently less than the recommended 200 counts per 100 ml[8,53] during most of the year for the continuous-flow plots; however, the intermittent-flow plot runoff gave acceptable values mainly during periods of subfreezing minimum temperatures (see Table B1 and Figure 3). Continuous freezing and thawing of the treatment slope surfaces at this time of the year was considered to be the main factor for the declines in effluent counts, based on the literature.[13,105,110]

Interferences from
nonfecal coliforms

118. The M-FC membrane filter test for fecal coliforms enumerates any microorganism that can grow sufficiently on the selective growth medium to produce a visible colony at an incubation temperature of 44.5°C, and can produce an acidic environment sufficient to produce blue coloration in the colonies.[7,82] In most cases this test enumerates primarily E. coli, the most common bacterium in the gut of man and most warm-blooded animals.[3,7] However, other related bacteria, especially Klebsiella and Enterobacter species and intermediate forms, can occasionally be enumerated at high numbers.[3,16,79,82] Enumeration of these related bacteria presents a dilemma. Klebsiella and Enterobacter, although commonly found at low numbers in feces, are capable of proliferation in natural, uncontaminated environments, especially in

association with vegetation.[12,77,78,111] The most common interfering isolate on fecal coliform plates is K. pneumoniae, an opportunistic pathogen. Although serious waterborne infections by this species are rare, most isolates obtained from the high temperature fecal coliform incubation are biochemically and serologically identical to clinical strains.[80,82,111] According to Dufour and Cabelli,[111] the relationship between thermotolerance and clinical origin is probably the result of natural selection, whereby disease-producing strains grow best at temperatures comparable with those in warm-blooded animals. Nevertheless, this should not necessarily indicate that apparently similar isolates will cause disease; disease potential should be based on epidemiological testing.[111]

119. Clinical strains of K. pneumoniae generally have three biochemical (IMViC) patterns, namely, --++, -+++, and ---+, the first pattern being by far the most numerous.[111] Based on the representative listing of isolates obtained on the M-FC fecal coliform plates (see Table B4), three out of four strains giving blue colonies were -+++, the least numerous of the clinical strains. Only two (out of 12) isolates fitted the most common pattern of --++. Thus perhaps in warmer environments, such as in the southern U. S. and tropical lowlands, a poorer relationship may exist between strains isolated by the fecal coliform test and strains of presumed clinical origin.

120. Enumeration problems created by abundant growth of K. pneumoniae on M-FC test plates were prevalent mainly at elevated temperatures (e.g., between 25° and 35°C) in lagoon wastewater, field plot runoff, and greenhouse model effluents (with and without the application of wastewater). This trend was also true for Enterobacter isolates and certain intermediate forms (e.g., isolate 118). Most field plot samples collected during the cooler weather (September through April) generally displayed mostly E. coli colonies on M-FC test plates, making fecal coliform enumerations much easier and more precise.

121. The K. pneumoniae generally displayed a deep blue colony color only when overcrowding occurred on an M-FC test plate. At acceptable colony counts (e.g., less than 100 colonies per plate), the colony color usually ranged from light blue to gray blue for possible positive

fecal coliform enumerations. Although most of the K. pneumoniae were pink to blue green at low numbers (negative background) on the M-FC plates, their abundance often resulted in a false negative count of accompanying E. coli colonies, resulting from a loss of blue color in the E. coli colonies. This color loss was also noted when strains of K. pneumoniae with blue colony color lost their color, often after incubation for less than an hour following ambient laboratory temperature exposure. The Enterobacter (e.g., E. cloacae) isolates frequently displayed a deep blue colony color at low plate counts. Although Enterobacter isolates were only sporadically a problem, E. cloacae colonies would readily overcrowd a plate with a spreading growth. Most of the Klebsiella and Enterobacter isolates possessed mucous colonies (see Tables 4 and B4; Figures 14, 15, and 16), whereas most of the E. coli colonies possessed crusty surfaces and contained a granular (mottled) and pink-centered blue colony color at a 10X magnification (see Table B3 and Figure 12). However, some E. coli colonies possessed a slightly mucous or watery consistency and lacked the characteristic pinkish center (see Figures 13 and 17).

Interferences from
false positive bacteria

122. Bacteria that form blue-colored colonies on M-FC test plates at low colony numbers (e.g., less than 200 colonies per plate) almost always produce β-galactosidase, an enzyme that breaks down the lactose in the M-FC medium; lactose fermentation leads to acid production, giving the blue color. A positive ONPG test indicates β-galactosidase activity. However, bacteria that are ONPG negative can also produce blue-colored colonies, but generally only at high colony number per test plate. Several such isolates, termed false positives, are morphologically and biochemically characterized in Table 5. Synergisms among more than one microorganism could be responsible, whereby one bacterium is capable of breaking down lactose but another bacterium is required for fermentation of the sugars to products that give the necessary pH for the blue-color reaction.

123. Acinetobacter calcoaceticus var. anitratus, in combination

with E. coli, was shown to cause a false positive reaction. Furthermore, when A. anitratus colonies were at high numbers (e.g., greater than 300 per plate) and E. coli colonies were at recommended counts (e.g., less than 100 per plate), the A. anitratus were frequently a deep blue color while the E. coli colonies were pink or off-blue (see Figure 18). Several other isolates formed blue colonies on M-FC test plates in the absence of E. coli colonies. Effluent test plates (see Figure 19b) showed two types of blue colonies that appeared to result from a synergistic reaction between a fecal coliform not keyed to E. coli (most large colonies on the effluent plates) and ONPG-negative blue pin colonies (see Tables 4 and 5). These isolates were obtained from a greenhouse model effluent while a morphologically distinct E. coli was being applied with deionized water; the E. coli colonies are shown on the influent (upper left) plate of Figure 19a. Despite the relatively large distances between the small false positive pin colonies and the large colonies, there was strong evidence that the pin colony bacterium had totally infiltrated the larger colonies. Overnight storage of the same plates (see Figure 19b) resulted in complete loss of color in both the false positive pin colonies and large lactose-fermenting colonies, whereas the E. coli remained blue.

124. Other bacteria commonly formed blue-colored colonies on M-FC test plates at high numbers, even if they were present as a monoculture. This reaction is due to their ability to grow on the selective M-FC medium, even though the bacteria cannot ferment lactose and establish the acidic conditions. Breakdown and fermentation of polysaccharides in their own cell walls can give a positive reaction. Comparative testing with phosphate buffer or sterile effluent water in the M-FC test dilution blanks indicated that microbial slimes in the runoff waters (e.g., from blue-green algae) were not the major cause for the false positive test reactions.

Methodology to Determine or Prevent Enumeration Problems

125. The presence of high numbers of interfering bacterial

colonies on M-FC test plates were found to cause varying degrees and types of interference. For example, a given K. pneumoniae isolate either caused color loss in adjacent E. coli colonies or showed little effect in enumeration of the E. coli. In other instances, high plate counts inhibited the growth of viable E. coli on the plates; this phenomenon was also observed when E. coli was the dominant isolate. In the majority of cases, poor membrane filter test results are caused by recording data from overcrowded test plates.[87] The analyst sometimes assumes that larger samples give more accurate results; at other times the counting of overcrowded plates results from a combination of poor estimation of numbers in a sample and time constraints. Manufacturers of membrane filter test supplies also often fail to stress the reasons for establishing an acceptable range of countable colony numbers. A maximum of 200 total colonies is recommended for the standard 47-mm-diam filters used for the M-FC test. The recommended range is 20-80 fecal coliform colonies per plate.[7,87,103]

126. The findings of this study demonstrate that the upper limit of 200 colonies per plate should not be surpassed. The total plate count should preferably be kept below 120 colonies, based on this study and the findings of other researchers.* Most of the observed problems could be eliminated by evaluating only the dilution plates displaying low total colony counts. Monitoring land runoff for fecal coliform bacteria may require the use of additional dilutions because of the often extreme and rapid fluctuations in total enumerable bacteria. This is especially true during warm weather. Fivefold dilutions are recommended since changes in positive counts should correspond with the dilution factor and false positive reactions have been noted to occur over even a fivefold dilution variance (see Figure 14). For example, if one dilution series give an average of 300 positive (blue) colonies on a plate while a fivefold greater dilution series gives an average of only 20, the larger value would be questionable and should indicate an interference

* Personal communication, Mr. W. G. Presswood, Chief Microbiologist, Gelman Instrument Co., Ann Arbor, Mich., May 1979.

problem. In this instance only the lower dilution replicate plates should be counted, even if the counts are less than the minimal recommended number of 20.

127. One noted problem with monitoring overland flow systems for fecal coliforms is that the occasionally very high background colony counts prevent the enumeration of E. coli at or even near levels accepted by the present criteria (i.e., 200 counts per 100-ml sample). There are several avenues to follow to determine if fecal coliform bacteria are being properly enumerated. One method would be to check for variances in counts with different dilution series and to use colony morphology as a helpful guide. However, severe growth hindrance or detection problems may necessitate the use of larger diameter membrane filters, which are commercially available upon request.* Spreading the colonies over a larger surface area allows E. coli to be more validly enumerated in a larger sample volume.

128. Present methodology generally uses the confirmed multiple tube fermentation (MPN) test as the comparative guide for evaluating efficacy of a new membrane filter test.** However, just as many or more serious enumeration problems are prevalent with the MPN procedures.[87-89] Since only a small number of samples were comparatively analyzed by the MPN fecal coliform technique (presumptive and confirmed tests), no valid evaluation is possible by this study. Because of the occasionally large variations in fecal coliform enumerations (both positive and negative) with overnight storage of collected samples at 4°C, immediate sample analysis is strongly recommended.

129. Comparison of the two-temperature modified M-FC test (including a preincubation at 35°C)[93] and the M-TEC membrane filter test[97] with the standard M-FC membrane filter test[5,7] showed that both new tests gave significantly higher fecal coliform enumerations, based on limited comparative testing. However, biochemical verification of isolates,

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* Personal communication, Mr. W. G. Presswood, Chief Microbiologist, Gelman Instrument Co., Ann Arbor, Mich., May 1979.

** Personal communication, Dr. Edwin Geldreich, U. S. Environmental Protection Agency, Cincinnati, Ohio, 11 May 1979.

using API-20E test strips, showed comparable numbers of verified E. coli colonies. This compariability suggests that both tests, each requiring a lower temperature preincubation, gave higher values mainly as a result of resuscitation of stressed cells. Color differentiation of positive colonies was more difficult with the M-TEC prccedure. However, since the M-TEC test gave consistently higher values whereas the modified M-FC test gave more sporadic results (e.g., occasionally lower values than the standard M-FC test), the M-TEC procedure should be evaluated further for its potential in enumerating stressed E. coli. The limited data for the M-TEC test are also insufficient to fully verify its documented better selectivity for E. coli.[97,108] Additional comparative testing of land runoff using these and other new membrane filter procedures is recommended.

Factors Affecting Treatment by Overland Flow Systems

Flow rate conditions

130. One consistent observation made at the Utica overland flow treatment site was the significantly better fecal coliform removal efficiency of the continuous-flow versus the intermittent-flow plots. Replicate plot monitoring showed an average elevenfold increase in effluent counts for the plots receiving wastewater intermittently, at a rate of 0.21 cm/hr for 6 hr/day for 5 days/week. Despite a lower flow rate of 0.11 cm/hr, the continuous-flow plots received 2.8 times the wastewater volume and bacterial biomass of the intermittent-flow plots each week. The differential treatment effect was most pronounced during the summer and fall months, when counts in effluents from the intermittent-flow plots were very high.

131. A tracer E. coli strain, doubly resistant to two antibiotics, was used to help clarify the flow rate phenomenon. The valid use of this strain as a bacterial tracer is well documented.[67,112] Two tests were performed on six treatment slopes at the two different flow rates by shifting the different flow rates among intermittent and continuous treatment plots. The experimental results showed that the plots receiving wastewater continuously for a long time period displayed the best fecal

coliform removal efficiencies. The twofold higher flow rate on the intermittent-flow plots, which had previously been treated continuously, resulted in approximately a tenfold increase in effluent fecal coliform numbers. The thousandfold higher numbers observed in effluents from plots that had always been run intermittently strongly suggested that other phenomena in addition to the increased flow rate were operative.

Biological conditions

132. Visual observation of the intermittent and the continuous treatment plots at Utica showed two noticeable differences: the lack of grass growth and the presence of a dark-colored surface organic layer on the upslope half of the continuously treated plots (see Figure 10b); better grass development and a conspicuous lack of the dark-colored layer was prevalent on the intermittent-flow plots (see Figure 10a). Examination of undisturbed surface soil samples from each of the two intermittent- and continuous-application plots (upslope and downslope regions) showed that the dark-colored layer was almost totally composed of the siliceous tests of pennate diatoms, which produced a relatively porous surface layer about 1 cm thick. This organic layer also harbored a much more numerous array of food chain microoganisms and arthropods.

133. At the time of soil observation, which was about 1 week following the final treatment of the plots with very high numbers of tracer E. coli and following weekend rains, the dominant predator microorganisms observed under the dissecting microscope were two ciliate protozoans, Paramecium and Vorticella. Several other protozoa were also present at low numbers. A sample from the upslope area of a continuous-flow plot showed enormous quantities (approximately $100/cm^2$) of large protozoa on the surface of the organic layer (Figure 20). However, the next highest occurrence was a soil sample from one of the intermittent-flow plots, although most of these individuals were inactive and appeared to be in a dormant state. Protozoa were infrequently observed in any of the downslope soil samples from the plots.

134. The significantly better fecal coliform treatment efficiency on the overland flow plots receiving continuous wastewater application at a reduced rate versus the intermittent-flow plots appears to be caused

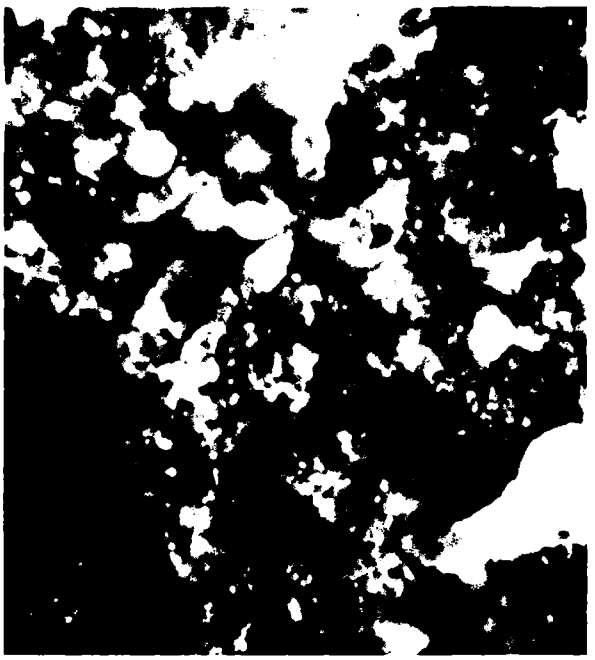

Figure 20. Surface organic layer showing many paramecia (whitish oval structures) from a continuous-flow field plot

by several factors. The major factors appear to be the increased detention of runoff water and a greater prevalence of predator-prey interactions on the continuous-flow plots. The increased detention could be caused by (a) the reduced influent flow rate, (b) the prevention of channeling of surface runoff by the diatomaceous surface soil layer, and (c) the sponge and filtration effect created by the presence of the porous surface layer. The diatomaceous soil layer observed under the dissecting microscope possessed a much higher effective surface area than that created by the grass stems and soil of the intermittent-flow plots. The predator-prey relationship also depends on entrapment and slower migration of the wastewater fecal coliform bacteria, which is elicited by the surface organic layer.

135. The importance of protozoan grazing on the maintenance of bacterial populations in aqueous environments is well documented.[113-116]

The decline of E. coli in wastewaters appears to be greatly influenced especially by the populations of ciliate protozoans,[115] whereas bacterial predation appears to be of much less importance.[114] Free-living ciliate protozoans proliferate only in aqueous environments. When the environment becomes devoid of water, these protozoans usually become inactive and form spherical structures with thick membranes; after prolonged desiccation, they can form angular-shaped cysts or resting cells. Although encysted protozoa can survive for prolonged periods, they are incapable of predation.[117] Thus, the numbers of active protozoan predators on the intermittent-flow plots should be much less than populations on the plots continuously receiving wastewater. This trend should be especially prevalent during the hot, dry summer months. Unfortunately, observations of the plot soil surfaces were made during a period of weekend rainfall, which perhaps allowed less variation between the different flow rates and durations. The random sampling indicated that protozoan populations fluctuate greatly on the plots; the higher numbers appeared to correspond to areas with higher bacterial populations. A detailed analysis would have been required for determining actual population densities.

136. In conclusion, the diatomaceous surface layer, which forms only under continuous or prolonged flow conditions, appears to greatly increase fecal coliform removal from overland runoff through bacterial entrapment and as a result of decreased channeling of the overland flow. The predatory protozoan populations should also increase under periods of continuous or prolonged flow. The increased residence time also allows increased protozoan predation on plots receiving continuous wastewater. Additionally, intermittent-flow conditions allow greater residency and increased soil contact of wastewater detritus, which may harbor the majority of viable fecal coliform bacteria. Therefore, actual growth of these bacteria should be better facilitated by short-term periods of nonapplication, such as overnight or over a weekend of drainage.

PART VI: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

137. The findings of this experimental study, combined with a thorough review of the literature, showed many problems in the use of the present (standard) M-FC membrane filter fecal coliform test for validly evaluating lagoon wastewater, overland flow system effluents, and most intermittent land runoff for recent fecal pollution. The observed problems seem to be especially severe on vegetated land surfaces since many fecal coliform and interfering bacteria grow naturally or persist in soil-plant environments, whereas dense vegetation prevents rapid die-off induced by desiccation of the soil surface. Persistently high field temperatures, such as those experienced in the southern states during the summer season, seem to further complicate the interpretation of test results. Elevated numbers of interfering bacteria, including certain fecal coliform bacteria that persist or grow in natural environments, were routinely isolated from lagoon wastewater and overland runoff during the warm summer months when daytime temperatures consistently approached 35°C. Natural selection for thermotolerant bacteria during extended periods of warm weather appears to be an important criterion for the observed test results on the overland flow treatment plots.

138. Elevated counts in effluent runoff were noted to persist through the cool fall and early winter periods until the first extensive subfreezing ambient conditions caused a noted population decline. However, the major isolate during the cooler weather was E. coli, which is considered to be the best bacterial indicator of recent fecal pollution and to be most indicative of the presence of viable enteric pathogens. The lowest effluent fecal coliform counts were encountered during the late winter and early spring periods. The continual freezing and thawing of the treatment plot surfaces, combined with low influent wastewater numbers, appeared to be responsible for this trend.

139. An interesting phenomenon occurred at the overland flow field site during the late spring and summer: higher fecal coliform

counts in effluent runoff than in influent wastewater collected contemporaneously. The findings suggested that certain stress factors in the lagoon, associated with algal blooms and/or alkaline pH, were responsible for the very low influent counts during this period. Additionally, the growth of E. coli, confounded by the positive and negative interferences created by the abundant interfering colonies on the test plates, appeared to be partly responsible for the very high effluent numbers. Similar observations were made during the application of lagoon wastewater to a greenhouse model. The wastewater factor inducing the growth appeared to be the movement of wastewater suspended solids in the overland flow. These solids could serve to protect viable E. coli and other fecal coliforms from harsh environmental conditions, including predation. Intermittent-flow conditions seemed to be most favorable for the growth of fecal coliforms (E. coli) on the overland flow system. Following drainage of the treatment slopes, detritus particles could come into intimate contact with the nutrient-rich organic layer on the surface of the overland flow plots; the static conditions presented by the lack of flowing water should also promote short-term growth in the adjacent soil. These additional bacteria could be released once flow conditions are resumed.

140. Another phenomenon observed on the overland flow field plots was the consistently much better fecal coliform removal obtained on plots receiving a continuous wastewater application versus those receiving the wastewater intermittently for 6 hr each day, 5 days/week. This phenomenon could be partly explained by the better growth conditions on the intermittent plots, induced by the longer detention and greater soil contact of suspended particles harboring the bacteria. However, predation appeared to be an important factor for fecal coliform reductions on the continuous-flow plots. The very high populations of ciliate protozoans observed in the field strongly suggested that predation by these protozoa was partly responsible for fecal bacterial declines; the observed protozoa remained active and proliferated only in an aqueous environment. Additionally, the observed presence of a conspicuous biological layer on the surface of the continuously treated plots appeared to

have increased the residency of the overland flow. This layer, composed primarily of diatom tests, would also serve as an effective bacterial filter, which should provide for easy scavenging by the predator organisms. A conspicuous organic surface layer was not observed on the plots receiving the intermittent wastewater application.

141. Overland flow treatment appeared to be very effective in removing fecal coliform bacteria from water under continuous-flow conditions. Poor treatment appears to be due mainly to the growth of fecal coliform bacteria on the overland flow treatment plots at elevated ambient temperatures during nonflow periods; short-term growth of E. coli was suggested during warm weather and subsequent cool weather. Good treatment, with fecal coliform removals in excess of 95 percent, is obtainable by maximizing the overland flow residence time (increasing the removal of suspended solids) and by stimulating natural predation. These factors are promoted by wastewater application at a slow, continuous rate. Since continuous-flow conditions are not favorable for good grass growth on the treatment plots and may promote an odor problem if wastewater high in nutrients is applied, perhaps an intermediate-flow condition would be most appropriate. For example, a wastewater application of 1-2 cm per hour during a 12- to 18-hr period each day (7 days/week) with nighttime drainage should preserve the conditions deemed necessary for good fecal coliform removal. Intermediate-flow conditions appeared to give much improved treatment than the intermittent conditions regularly used on the plots with the weekend drying.

Recommendations

142. The standard M-FC membrane filter fecal coliform test can be used for monitoring the quality of some but not all land runoff. Several modified M-FC testing procedures and new tests (e.g., the M-TEC test) should be compared to help determine which procedure gives the best indication of recent fecal contamination. However, the results of this study indicate that a selective testing procedure for E. coli, rather than a fecal coliform test, is more valid for evaluating the

fecal pollution potential of land runoff. Colony verification by biochemical testing is recommended whenever a problem is suspected.

143. Environmental stress may cause low counts, and lagoon wastewater appears to be subject to many stress factors. Several stress-recovery modifications to the M-FC test are available and each modification may be best for different forms of stress. The standard MPN test should also be considered in the test evaluations. Such evaluations should initially be performed during each season of the first year, and performed thereafter whenever a problem is indicated by unusual test results.

144. Overnight sample storage at 4°C can often lead to drastically different fecal coliform counts. Therefore, immediate sample testing is recommended. If large differences occur after overnight storage, the presence of large numbers of stressed microorganisms should be considered.

145. The use of at least six fivefold dilutions is suggested for fecal coliform analysis with the membrane filter tests since interfering (e.g., false positive) colonies can cause color interference fluctuations at this dilution range. This study strongly recommends avoiding overcrowded membrane filter test plates because of the positive and negative interference problems created when different or the same bacterial colonies are proximate. Total colony counts should never exceed 200 (preferably 120).

146. If background colony counts are very high, excluding the development of E. coli colonies, the use of a larger diameter membrane filter is suggested; a comparable ratio of colony number to filter size should be maintained. In instances of excessive suspended solids or background colony growth, the use of membrane filter tests may not be possible and the use of the MPN test (presumptive and confirmed steps) may be necessary.

147. Fecal coliform removal from wastewater should be promoted by slow, continuous-flow conditions on overland flow treatment plots. The recommended rate for best treatment is daily application at 1-2 cm/hr for 12-24 hr. A weekend dry spell favors fecal coliform growth on the

treatment plots and inhibits the active proliferation of predator protozoa; the development of a biological surface layer only under continuous-flow conditions, facilitating bacterial removal, indicates that other phenomena are also involved.

148. The study findings suggest that the efficient removal of suspended organic detritus in settling ponds, prior to overland flow treatment, would favor low fecal coliform counts in effluent runoff from the overland flow system. However, slow- and continuous-flow conditions seem to be best for the removal of suspended detritus and their associated viable bacterial populations.

REFERENCES

1. Chordash, R. A. and Insalata, N. F., "Incidence and Pathological Significance of Escherichia coli and Other Sanitary Indicator Organisms in Food and Water," Food Technology, Vol 32, No. 10, Oct 1978, pp 54-63.

2. Pelczar, M. J. and Reid, R. D., Microbiology, 2nd Ed., McGraw-Hill, N. Y., 1965, pp 426-433.

3. Dufour, A. P., "Escherichia coli: The Fecal Coliform," American Society for Testing and Materials, Special Technical Publication 635, 1977, pp 48-58.

4. Gallagher, T. P. and Spino, D. F., "The Significance of Numbers of Coliform Bacteria as an Indicator of Enteric Pathogens," Water Research, Vol 2, 1968, pp 169-175.

5. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 14th Ed., Washington, D. C., 1975.

6. ORSANCO Water Users Committee, "Total Coliform: Fecal Coliform Ratio for Evaluation of Raw Water Bacterial Quality," Journal Water Pollution Control Federation, Vol 43, No. 4, Apr 1971, pp 630-640.

7. Bordner, R., Winter, J., and Scarpino, P., Microbiological Methods for Monitoring the Environment: Water and Wastes, EPA-600/8-78-017, Dec 1978, Environmental Monitoring and Support Laboratory, U. S. EPA, Cincinnati, Ohio.

8. Geldreich, E. E., "Applying Bacteriological Parameters to Recreational Water Quality," Journal of the American Water Works Association, Vol 62, No. 2, Feb 1970, pp 113-120.

9. Geldreich, E. E., Clark, H. F., and Huff, C. B., "A Study of Pollution Indicators in a Waste Stabilization Pond," Journal Water Pollution Control Federation, Vol 36, No. 11, Nov 1964, pp 1372-1379.

10. Kibbey, H. J., Hagedorn, C., and McCoy, E. L., "Use of Fecal Streptococci as Indicators of Pollution in Soil," Applied and Environmental Microbiology, Vol 35, No. 4, Apr 1978, pp 711-717.

11. Hunt, P. G., Peters, R. E., Sturgis, T. C., and Lee, C. R., "Reliability Problems with Indicator Organisms for Monitoring Overland Flow Treated Waste Water Effluent," Journal of Environmental Quality, Vol 8, No. 3, 1979, pp 301-304.

12. Geldreich, E. E., Kenner, B. A., and Kabler, P. W., "Occurrence of Coliforms, Fecal Coliforms, and Streptococci on Vegetation and Insects," Applied Microbiology, Vol 12, No. 1, Jan 1964, pp 63-69.

13. Gerba, C. P., Wallis, C., and Melnick, J. L., "Fate of Wastewater Bacteria and Viruses in Soil," Journal of the Irrigation and Drainage Division, American Society of Civil Engineers, Vol 101, IR3, Sep 1975, pp 157-174.

14. Schaub, S. A., Kenyon, K. F., Bledsoe, B., and Thomas, R. E., "Evaluation of the Overland Runoff Mode of Land Wastewater Treatment for Virus Removal," Applied and Environmental Microbiology, Vol 39, No. 1, Jan 1980, pp 127-134.

15. Goyal, S. M., Zerda, K. S., and Gerba, C. P., "Concentration of Coliphages from Large Volumes of Water and Wastewater," Applied and Environmental Microbiology, Vol 39, No. 1, Jan 1980, pp 85-91.

16. Geldreich, E. E., Best, L. C., Kenner, B. A., and VanDonsel, D. J., "The Bacteriological Aspects of Stormwater Pollution," Journal Water Pollution Control Federation, Vol 40, No. 11, Nov 1968, pp 1861-1872.

17. Skinner, Q. D., Adams, J. C., Rechard, P. A., and Beetle, A. A., "Effect of Summer Use of a Mountain Watershed on Bacterial Water Quality," Journal of Environmental Quality, Vol 3, No. 4, 1974, pp 329-335.

18. Doran, J. W. and Linn, D. M., "Bacteriological Quality of Runoff Water from Pastureland," Applied and Environmental Microbiology, Vol 37, No. 5, May 1979, pp 985-991.

19. Mitchell, D. O. and Starzyk, M. J., "Survival of Salmonella and Other Indicator Microorganisms," Canadian Journal of Microbiology, Vol 21, No. 9, Sep 1975, pp 1420-1421.

20. VanDonsel, D. J. and Geldreich, E. E., "Relationships of Salmonellae to Fecal Coliforms in Bottom Sediments," Water Research, Vol 5, 1971, pp 1079-1087.

21. Smith, R. J., Twedt, R. M., and Flanigan, L. K., "Relationships of Indicator and Pathogenic Bacteria in Stream Waters," Journal Water Pollution Control Federation, Vol 45, No. 8, Aug 1973, pp 1736-1745.

22. Fair, J. F. and Morrison, S. M., "Recovery of Bacterial Pathogens from High Quality Surface Water," Water Resource Research, Vol 3, No. 3, 1967, pp 799-803.

23. McFeters, G. A., Bissonnette, G. K., Jezeski, J. J., Thomson, C. A., and Stuart, D. G., "Comparative Survival of Indicator Bacteria and Enteric Pathogens in Well Water," Applied Microbiology, Vol 27, No. 5, May 1974, pp 823-829.

24. VanDonsel, D. J., Geldreich, E. E., and Clarke, N. A., "Seasonal Variations in Survival of Indicator Bacteria in Soil and Their Contribution to Storm-Water Pollution," Applied Microbiology, Vol 15, No. 6, Nov 1967, pp 1362-1370.

25. Bitton, G. and Mitchell, R., "Protection of E. coli by Montmorillonite in Seawater," Journal of the Environmental Engineering Division, Proc. American Society of Civil Engineers, Vol 100, No. EE6, Dec 1974, pp 1310-1313.

26. Faust, M. A., Aotaky, A. E., and Hargadon, M. T., "Effect of Physical Parameters on the In Situ Survival of Escherichia coli MC-6 in an Estuarine Environment," Applied Microbiology, Vol 30, No. 5, Nov 1975, pp 800-806.

27. Gudding, R. and Krogstad, O., "The Persistence of Escherichia coli and Salmonella typhimurium in Fine-Grained Soil," Acta Agriculturae Scandinavica, Vol 25, No. 4, 1975, pp 285- 88.

28. Gorden, R. W. and Fliermans, C. B., "Survival and Viability of Escherichia coli in a Thermally Altered Reservoir," Water Research, Vol 12, 1978, pp 343-352.

29. Zibilske, L. M. and Weaver, R. W., "Effect of Environmental Factors on Survival of Salmonella typhimurium in Soil," Journal of Environmental Quality, Vol 7, No. 4, 1978, pp 593-597.

30. Hanes, N. B., Rohlich, G. A., and Sarles, W. B., "Effect of Temperature on the Survival of Indicator Bacteria in Water," New England Water Works Association Journal, Vol 80, 1965, pp 6-18.

31. Gameson, A. L. H. and Saxon, J. R., "Field Studies on Effect of Daylight on Mortality of Coliform Bacteria," Water Research, Vol 1, 1967, pp 279-295.

32. Jones, G. E., "The Fate of Freshwater Bacteria in the Sea," Developments in Industrial Microbiology, Vol 12, Chap. 16, 1971, pp 141-151.

33. Bell, R. G., "Persistence of Fecal Coliform Indicator Bacteria on Alfalfa Irrigated with Municipal Sewage Lagoon Effluent," Journal of Environmental Quality, Vol 5, No. 1, 1976, pp 39-42.

34. Bell, R. G. and Bole, J. B., "Elimination of Fecal Coliform Bacteria from Reed Canarygrass Irrigated with Municipal Sewage Lagoon Effluent," Journal of Environmental Quality, Vol 5, No. 4, 1976, pp 417-418.

35. Bell, R. G., Wilson, D. B., and Dew, E. J., "Feedlot Manure Top Dressing for Irrigated Pasture: Good Agricultural Practice or a Health Hazard?," Bulletin of Environmental Contamination and Toxicology, Vol 16, No. 5, Nov 1976, pp 536-540.

36. Gerba, C. P. and McLeod, J. S., "Effect of Sediments on the Survival of Escherichia coli in Marine Waters," Applied and Environmental Microbiology, Vol 32, No. 1, Jul 1976, pp 114-120.

37. Dazzo, F., Smith, P., and Hubbell, D., "The Influence of Manure Slurry Irrigation on the Survival of Fecal Organisms in Scranton Fine Sand," Journal of Environmental Quality, Vol 2, No. 4, 1973, pp 470-473.

38. Lance, J. C. and Whisler, F. D., "The Effect of Increasing the Organic Carbon Content of Sewage on Nitrogen, Carbon, and Bacteria Removal and Infiltration in Soil Columns," Hydrology and Water Resources in Arizona and the Southwest, Vol 5, 1975, pp 57-65.

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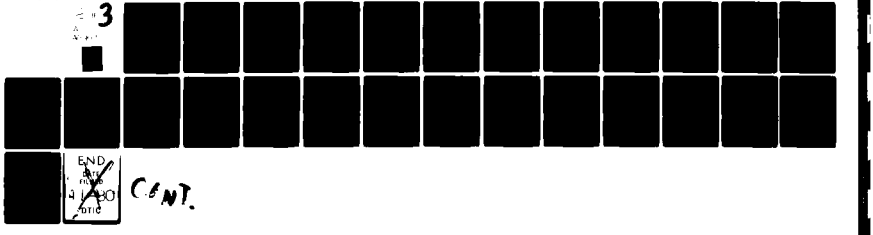

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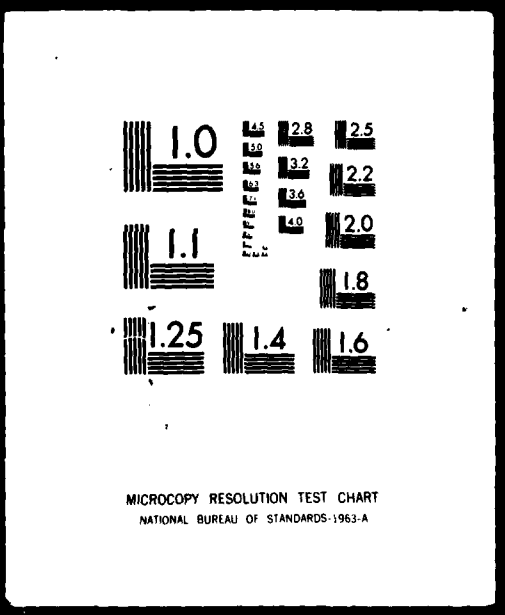

39. Mitchell, R., "Factors Affecting the Decline of Non-Marine Microorganisms in Seawater," Water Research, Vol 2, 1968, pp 535-543.

40. Mallmann, W. L. and Litsky, W., "Survival of Selected Enteric Organisms in Various Types of Soil," American Journal of Public Health, Vol 41, No. 1, Jan 1951, pp 38-44.

41. McFeters, G. A. and Stuart, D. G., "Survival of Coliform Bacteria in Natural Waters: Field and Laboratory Studies with Membrane-Filter Chambers," Applied Microbiology, Vol 24, No. 5, Nov 1972, pp 805-811.

42. Wu, S.-Y. and Klein, D. A., "Starvation Effects on Escherichia coli and Aquatic Bacterial Responses to Nutrient Addition and Secondary Warming Stresses," Applied and Environmental Microbiology, Vol 31, No. 2, Feb 1976, pp 216-220.

43. Seidler, R. J. and Starr, M. P., "Factors Affecting the Intracellular Parasitic Grown of Bdellovibrio bacteriovorus Developing Within Escherichia coli," Journal of Bacteriology, Vol 97, No. 2, Feb 1969, pp 912-923.

44. Gray, E. A., "Survival of Escherichia coli in Stream Water in Relation to Carbon Dioxide and Plant Photosynthesis," Journal of Applied Bacteriology, Vol 39, 1975, pp 4754.

45. Cuthbert, W. A., Panes, J. J., and Hill, E. C., "Survival of Bacterium coli and Streptococcus faecalis in Soil," Journal of Applied Bacteriology, Vol 18, 1955, pp 408-414.

46. Hanes, N. B., Sarles, W. B., and Rohlich, G. A., "Dissolved Oxygen and Survival of Coliform Organisms and Enterococci," Journal of American Water Works Association, Vol 56, Apr 1964, pp 441-446.

47. Klock, J. W., "Survival of Coliform Bacteria in Wastewater Treatment Lagoons," Journal Water Pollution Control Federation, Vol 43, No. 10, Oct 1971, pp 2071-2083.

48. Sweeten, J. M. and Reddell, D. L., "Nonpoint Sources: State-of-the-Art Overview," Transactions of the American Society of Agricultural Engineers, Vol 21, No. 3, 1978, pp 474-483.

49. Stephenson, G. R. and Street, L. V., "Bacterial Variations in Streams from a Southwest Idaho Rangeland Watershed," Journal of Environmental Quality, Vol 7, No. 1, 1978, pp 150-157.

50. Kunkle, S. H., "Concentrations and Cycles of Bacterial Indicators in Farm Surface Runoff," Proceedings, Cornell Agricultural Waste Management Conference, Syracuse, N. Y., Jan 970, pp 19-21.

51. Weidner, R. B., Christianson, A. G., Weibel, S. R., and Robeck, G. G., "Rural Runoff as a Factor in Stream Pollution," Journal Water Pollution Control Federation, Vol 41, No. 3, Mar 1969, pp 377-384.

52. Meiman, J. R. and Kunkle, S. H., "Land Treatment and Water Quality Control," Journal of Soil and Water Conservation, Vol 22, No. 2, 1967, pp 67-70.

53. U. S. Department of the Interior, "Water Quality Criteria," Report of the National Technical Advisory Committee to the Secretary of the Interior, 1968, Federal Water Pollution Control Administration, Washington, D. C.

54. Burwell, R. E., Schuman, G. E., Piest, R. F., Spomer, R. G., and McCalla, T. M., "Quality of Water Discharged from Two Agricultural Watersheds in Southwestern Iowa," Water Resources Research, Vol 10, No. 2, Apr 1974, pp 359-365.

55. Smith, J. H. and Douglas, C. L., "Microbiological Quality of Surface Drainage Water From Three Small Irrigated Watersheds in Southern Idaho," Journal of Environmental Quality, Vol 2, No. 1, 1973, pp 110-112.

56. Dornbush, J. N., Andersen, J. R., and Harms, L. L., "Quantification of Pollutants In Agricultural Runoff," EPA-660/2-74-005, Feb 1974, U. S. Environmental Protection Agency, Office of Research and Development, Washington, D. C.

57. Benarde, M. A., "Land Disposal and Sewage Effluent: Appraisal of Health Effects of Pathogenic Organisms," Journal American Water Works Association, Vol 65, No. 6, Jun 1973, pp 432-440.

58. Weaver, D. E., Mang, J. L., Galke, W. A., and Love, G. J., "Potential for Adverse Health Effects Associated with the Application of Wastewaters or Sludges to Agricultural Lands," Land as a Waste Management Alternative, R. C. Loehr, ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Mich., 1977, pp 363-389.

59. Burge, W. D. and Marsh, P. B., "Infectious Disease Hazards of Landspreading Sewage Wastes," Journal of Environmental Quality, Vol 7, No. 1, 1978, pp 1-9.

60. Katzenelson, E., Buium, I., and Shuval, H. I., "Risk of Communicable Disease Infection Associated with Wastewater Irrigation in Agricultural Settlements," Science, Vol 194, No. 4268, Nov 1976, pp 944-946.

61. Morrison, S. M. and Martin, K. L., "Pathogen Survival in Soils Receiving Waste," Land as a Waste Management Alternative, R. C. Loehr, ed., Ann Arbor Science Publishers, Inc., Ann Arbor, Mich., 1977, pp 371-389.

62. Foster, D. H. and Engelbrecht, R. S., "Microbial Hazards in Disposing of Wastewater on Soil," Recycling Treated Municipal Wastewater and Sludge through Forest and Cropland, W. E. Sopper and L. T. Kardos, eds. The Pennsylvania State University Press, University Park, Pa., 1973, pp 247-270.

63. Hoeppel, R. E., Hunt, P. G., and Delaney, T. B., Jr., "Wastewater Treatment on Soils of Low Permeability," Misc. Paper Y-74-2, Jul 1974, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

64. Lee, C. R., Hunt, P. G., Hoeppel, R. E., Carlson, C. A., Delaney, T. B., Jr., and Gordon, R. N., "Highlights of Research on Overland Flow for Advanced Treatment of Wastewater," Misc. Paper Y-76-6, Nov 1976, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

65. Krone, R. B., "The Movement of Disease Producing Organisms Through Soils," Municipal Sewage Effluent for Irrigation, C. E. Wilson and F. E. Beckett, eds., Agricultural Engineering Department, Louisiana Polytechnic Institute, Ruston, La., 1968, pp 75-105.

66. Gilbert, R. G., Gerba, C. P., Rice, R. C., Bouwer, H., Wallis, C., and Melnick, J. L., "Virus and Bacteria Removal from Wastewater by Land Treatment," Applied and Environmental Microbiology, Vol 32, No. 3, Sep 1976, pp 333-338.

67. Rahe, T. M., Hagedorn, C., McCoy, E. L., and Kling, G. F., "Transport of Antibiotic-resistant Escherichia coli Through Western Oregon Hillslope Soils Under Conditions of Saturated Flow," Journal of Environmental Quality, Vol 7, No. 4, 1978, pp 487-494.

68. Hendricks, C. W. and Morrison, S. M., "Multiplication and Growth of Selected Enteric Bacteria in Clear Mountain Stream Water," Water Research, Vol 1, 1967, pp 567-576.

69. Bell, R. G. and Bole, J. B., "Elimination of Fecal Coliform Bacteria from Soil Irrigated with Municipal Sewage Lagoon Effluent," Journal of Environmental Quality, Vol 7, No. 2, 1978, pp 193-196.

70. Kristensen, K. K. and Bonde, G. J., "The Current Status of Bacterial and Other Pathogenic Organisms in Municipal Wastewater and Their Potential Health Hazards with Regard to Agricultural Irrigation," Wastewater Renovation and Reuse, F. M. D'Itri, ed., Marcel Dekker, Inc., New York, N. Y., 1977, pp 387-419.

71. Sadovski, A. Y., Fattal, B., and Goldberg, D., "Microbial Contamination of Vegetables Irrigated with Sewage Effluent by the Drip Method," Journal of Food Protection, Vol 41, No. 5, May 1978, pp 336-340.

72. Grunnet, K. and Moller, J., "Changes in Biological Parameters on Grass, Hay, and in Silage Following Irrigation with Domestic Sewage," Progress in Water Technology, Vol 11, Nos. 4/5, 1979, pp 139-146.

73. Standridge, J. H., Delfino, J. J., Kleppe, L. B., and Butler, R., "Effect of Waterfowl (Anas platyrhynchos on Indicator Bacteria Populations in a Recreational Lake in Madison, Wisconsin," Applied and Environmental Microbiology, Vol 38, No. 3, Sep 1979, pp 547-550.

74. Stuart, D. G., Bissonnette, G. K., Goodrich, T. D., and Walter, W. G., "Effects of Multiple Use on Water Quality of High-Mountain Watersheds: Bacteriological Investigations of Mountain Streams," Applied Microbiology, Vol 22, No. 6, Dec 1971, pp. 1048-1054.

75. Fraser, M. H., Reid, W. B., and Malcolm, J. F., "The Occurrence of Coli-Aerogenes Organisms on Plants," Journal of Applied Bacteriology, Vol 19, No. 2, 1956, pp 301-309.

76. Walter, W. G. and Bottman, R. P., "Microbiological and Chemical Studies of an Open and Closed Watershed," Journal of Environmental Health, Vol 30, Sep-Oct 1967, pp 157-163.

77. Seidler, R. J., Morrow, J. E., and Bagley, S. T., "Klebsielleae in Drinking Water Emanating from Redwood Tanks," Applied and Environmental Microbiology, Vol 33, No. 4, Apr 1977, pp 893-900.

78. Talbot, H. W., Jr. and Seidler, R. J., "Gas Chromatographic Analysis of In Situ Cyclitol Utilization by Klebsielleae Growing in Redwood Extracts," Applied and Environmental Microbiology, Vol 38, No. 4, Oct 1979, pp 599-605.

79. Duncan, D. W. and Razzell, W. E., "Klebsiella Biotypes Among Coliforms Isolated from Forest Environments and Farm Produce," Applied Microbiology, Vol 24, No. 6, Dec 1972, pp 933-938.

80. Brown, C. and Seidler, R. J., "Potential Pathogens in the Environment: Klebsiella pneumoniae, a Taxonomic and Ecological Enigma," Applied Microbiology, Vol 25, No. 6, Jun 1973, pp 900-904.

81. Matsen, J. M, Spindler, J. A., and Blosser, R. O., "Characterization of Klebsiella Isolates from Natural Receiving Waters and Comparison with Human Isolates," Applied Microbiology, Vol 28, No. 4, Oct 1974, pp 672-678.

82. Bagley, S. T. and Seidler, R. J., "Significance of Fecal Coliform-Positive Klebsiella," Applied and Environmental Microbiology, Vol 33, No. 5, May 1977, pp 1141-1148.

83. Ishiguro, N., Oka, C., and Sato, G., "Isolation of Citrate-Positive Variants of Escherichia coli from Domestic Pigeons, Pigs, Cattle and Horses," Applied and Environmental Microbiology, Vol 36, No. 2, Aug 1978, pp 217-222.

84. Geldreich, E. E., Huff, C. B. Bordner, R. H., Kabler, P. W., and Clark, H. F., "The Faecal Coli-Aerogenes Flora of Soils from Various Geographical Areas," Journal of Applied Bacteriology, Vol 25, No. 1, 1962, pp 87-93.

85. Kasweck, K. L. and Fliermans, C. B., "Lactose Variability of Escherichia coli in Thermally Stressed Reactor Effluent Waters," Applied and Environmental Microbiology, Vol 36, No. 5, Nov 1978, pp 739-746.

86. Murooka, Y. and Harada, T., "Expansion of the Host Range of Coliphage P1 and Gene Transfer from Enteric Bacteria to Other Gram-Negative Bacteria," Applied and Environmental Microbiology, Vol 38, No. 4, Oct 1979, pp 754-757.

87. Geldreich, E. E., Jeter, H. L., and Winter, J. A., "Technical Considerations in Applying the Membrane Filter Procedure," Health Laboratory Science, Vol 4, No. 2, Apr 1967, pp 113-125.

88. Geldreich, E. E., Allen, M. J., and Taylor, R. H., "Interferences to Coliform Detection in Potable Water Supplies," Evaluation of the Microbiology Standards for Drinking Water, C. W. Hendricks, ed., EPA-570/9-78-006, Aug 1978, Office of Drinking Water, U. S. EPA, Washington, D. C., pp 13-20.

89. Bordner, R. H., Frith, C. F. and Winter, J. A., eds., "Proceedings of the Symposium on the Recovery of Indicator Organisms Employing Membrane Filters," EPA-600/9-77-024, Sep 1977, Environmental Monitoring and Support Laboratory, U. S. EPA, Cincinnati, Ohio.

90. Presswood, W. G. and Strong, D. K., "Modification of M-FC Medium by Eliminating Rosolic Acid," Applied and Environmental Microbiology, Vol 36, No. 1, Jul 1978, pp 90-94.

91. Buras, N. and Kott, Y., "A Simple Test for Differentiation Between E. coli and A. aerogenes," Water Research, Vol 3, 1969, pp 973-978.

92. Rose, R. E., Geldreich, E. E., and Litsky, W., "Improved Membrane Filter Method for Fecal Coliform Analysis," Applied Microbiology, Vol 29, No. 4, Apr 1975, pp 532-536.

93. Green, B. L., Clausen, E. M., and Litsky, W., "Two-Temperature Membrane Filter Method for Enumerating Fecal Coliform Bacteria from Chlorinated Effluents," Applied and Environmental Microbiology, Vol 33, No. 6, Jun 1977, pp 1259-1264.

94. Stuart, D. G., McFeters, G. A., and Schillinger, J. E., "Membrane Filter Technique for the Quantification of Stressed Fecal Coliforms in the Aquatic Environment," Applied and Environmental Microbiology, Vol 34, No. 1, Jul 1977, pp 42-46.

95. Martin, S. E., Flowers, R. S., and Ordal, Z. J., "Catalase: Its Effect on Microbial Enumeration," Applied and Environmental Microbiology, Vol 32, No. 5, Nov 1976, pp 731-734.

96. Lonsane, B. K., Parhad, N. M., and Rao, N. U., "Effect of Storage Temperature and Time on the Coliforms in Water Samples," Water Research, Vol 1, 1967, pp 309-316.

97. Dufour, A. P., Strickland, E. R., and Cabelli, V. J., "A Membrane Filter Procedure for Enumerating Thermotolerant E. coli," Proceedings, 9th National Shellfish Sanitation Workshop, D. S. Wilt, ed., U. S. Food and Drug Administration, 25 Jun 1975, Charleston, S. C.

98. Dufour, A. P. and Strickland, E., "A Single Step Procedure for Enumerating E. coli on Membrane Filters," Abstracts of the Annual Meeting of the American Society for Microbiology, 79th Annual Meeting, Los Angeles, Calif., May 1979, p 229.

99. Munoz, E. F. and Silverman, M. P., "Rapid, Single-Step Most-Probable-Number Method for Enumerating Fecal Coliforms in Effluents from Sewage Treatment Plants," Applied and Environmental Microbiology, Vol 37, No. 3, Mar 1979, pp 527-530.

100. Warren, L. S., Benoit, R. E., and Jessee, J. A., "Rapid Enumeration of Fecal Coliforms in Water by a Colorimetric B-Galactosidase Assay," Applied and Environmental Microbiology, Vol 35, No. 1, Jan 1978, pp 136-141.

101. U. S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-625/6-74-003, 1974, National Environmental Research Center, Cincinnati, Ohio.

102. Taylor, J. M., Epstein, E., Burge, W. D., Chaney, R. L., Menzies, J. D., and Sikora, L. J., "Chemical and Biological Phenomena Observed with Sewage Sludges in Simulated Soil Trenches," Journal of Environmental Quality, Vol 7, No. 4, 1978, pp 477-482.

103. Van Dyne, D. L. and Gilbertson, C. B., "Estimating U. S. Livestock and Poultry Manure Nutrient Production," ESCS-12, 1978, U. S. Department of Agriculture.

104. Millipore Corp., "Biological Analysis of Water and Wastewater," Application Manual, AM302, Bedford, Mass., 1973.

105. Maxcy, R. B., "Non-Lethal Injury and Limitations of Recovery of Coliform Organisms on Selective Media," Journal of Milk Food Technology, Vol 33, 1970, pp 445-448.

106. Egan, A. F., "Enumeration of Stressed Cells of Escherichia coli," Canadian Journal of Microbiology, Vol 25, 1979, pp 116-118.

107. Skerry, G. P. and Parker, C. D., "Development of an Improved Quantitative Relationship Between Bacterial Die-Off, Design and Operational Factors for Anaerobic-Aerobic and Maturation Type Lagoon Systems," Progress in Water Technology, Vol 11, No. 4/5, 1979, pp 427-443.

108. Pagel, J. E. and Vlassoff, L. T., "Determination of Performance Characteristics for Fecal Coliform Enumeration Procedures," Abstracts of the Annual Meeting of the American Society for Microbiology, 79th Annual Meeting, Los Angeles, Calif., May 1979.

109. Davis, E. M., "Maximum Utilization of Water Resources in a Planned Community: Bacterial Characteristics of Stormwaters in Developing Rural Areas," EPA 600/2-79-050f, Aug 1979, Municipal Environmental Research Laboratory, U. S. EPA, Cincinnnati, Ohio.

110. Straka, R. P. and Stokes, J. L., "Metabolic Injury to Bacteria at Low Temperatures," Journal of Bacteriology, Vol 78, 1959, pp 181-185.

111. Dufour, A. P. and Cabelli, V. J., "Characteristics of Klebsiella from Textile Finishing Plant Effluents," Journal Water Pollution Control Federation, Vol 48, No. 5, May 1976, pp 872-879.

112. Hagedorn, C. and McCoy, E. L., "Soil Suitability for On-Site Waste Disposal: Development of Genetically Marked Escherichia coli Strains as Tracers of Subsurface Water Flow," WRRI-63, Nov 1979, Office of Water Research and Technology, U. S. Dept. of the Interior, Water Resources Institute, Oregon State Univ., Corvallis, Oreg.

113. Gude, H., "Grazing by Protozoa as Selection Factor for Activated Sludge Bacteria," Microbial Ecology, Vol 5, 1979, pp 225-237.

114. Enzinger, R. M. and Cooper, R. C., "Role of Bacteria and Protozoa in the Removal of Escherichia coli from Estuarine Waters," Applied and Environmental Microbiology, Vol 31, No. 5, May 1976, pp 758-763.

115. Curds, C. R. and Fey, G. J., "The Effect of Ciliated Protozoa on the Fate of Escherichia coli in the Activated Sludge Process," Water Research, Vol 7, 1969, pp 853-867.

116. Tate, R. L., III, "Cultural and Environmental Factors Affecting the Longevity of Escherichia coli in Histosols," Applied and Environmental Microbiology, Vol 35, No. 5, May 1978, pp 925-929.

117. Kudo, R. R., Protozoology, 5th ed., Charles C. Thomas Publisher, Springfield, Ill., 1971, pp 902-906.

Table 1

Response of Greenhouse Overland Runoff Model 2 to the Application of Deionized Water Containing Tracer (Streptomycin-Resistant) E. coli*

Day	Julian Date	Influent E. coli No./100 ml	Effluent E. coli No./100 ml	Reduction in Concentration, %	Mass Removal Efficiency, %
Wednesday	178	47,016,700	3,982,500	91.2	94.6
Thursday	179	41,377,800	5,709,400	86.2	91.5
Monday	183	40,133,300	--	--	--
Tuesday	184	51,450,000	5,412,000	89.5	95.0
Thursday	186	38,383,300	5,320,000	86.1	92.5
Tuesday	191	41,766,700	7,000,000	83.2	88.5
Thursday	193	37,411,100	5,819,000	84.4	90.2
Tuesday	198	47,522,200	--	--	--
Thursday	200	45,344,400	7,631,800	83.2	88.2
Tuesday	205	34,922,200	8,596,300	75.4	82.1
Thursday	207	53,822,200	3,739,400	93.1	94.7
Tuesday	212	45,325,000	8,258,300	81.8	87.0
Tuesday	219	41,144,400	4,340,700	89.5	92.3
Thursday	221	45,888,900	6,132,000	86.6	89.7
Tuesday	226	44,800,000	3,315,000	92.6	94.6
Thursday	228	54,366,700	6,030,000	88.9	91.4
Tuesday	233	38,640,000	3,116,700	91.9	94.2
Thursday	235	44,333,300	1,837,300	95.9	96.9
	Averages	41,628,600	5,390,000	87.1	91.7

* Application with 1.25 cm water plus tracer bacterium over a 6-hr period, 5 days/week.

Table 2

Application of Lagoon Wastewater to a Greenhouse Overland Runoff Model at Two Different Rates and Determination of Fecal Coliform Response

Wastewater Influent Rate 1.25 cm per 6 hr				Wastewater Influent Rate 1.25 cm per 18 hr			
		Fecal Coliform Count No./100 ml				Fecal Coliform Count No./100 ml	
Day	Julian Date	Influent	Effluent	Day	Julian Date	Influent	Effluent
Tuesday	331	655	3,260	Thursday	347	35	300
Wednesday	332	300	280	Monday	351	0	450
Thursday	333	75	390	Tuesday	352	2900	100
Monday	337	4000	TNTC*	Wednesday	353	240	215
Tuesday	338	5600	16,750	Thursday	354	5	130
Wednesday	339	1700	6,700	Wednesday	360	10	65
Thursday	340	25	3,200	Wednesday	002	915	35
Monday	344	2300	70,000	Thursday	003	65	490
Tuesday	345	60	11,000	Friday	004	10	185
Wednesday	346	5	1,000**	Monday	007	4165	†
				Tuesday	008	310	135
				Wednesday	009	20	45
				Thursday	010	10	25
	Averages	1635	14,000			668	181

* TNTC = too numerous to count.

** First day of 18-hr rate appears to represent a transition period; not included in averages.

† No runoff occurred.

Table 3

Elution of Cattle Manure from the Surface of the Upper Half of Greenhouse Overland Runoff Model 3*

Day	Julian Date	Total Fecal Coliforms Estimated in Applied Manure**	Fecal Coliform Concentration in Runoff Water, No./100 ml†				Total Fecal Coliforms Estimated in Runoff	Mass Removal Efficiency, %
			Initial	1-2 hr	4 hr	Average		
Tuesday	205	320.0×10^6	270	130	30	140	65,800	>99.9
Thursday	207	123.3×10^6	1085	565	25	560	291,200	99.8
Tuesday	212	245.0×10^6	--	770	155	460	202,400	99.9
Thursday	214	333.3×10^6	1200	745	65	670	355,100	99.9
Tuesday	219	21.7×10^6	30	125	45	65	33,200	99.8
Thursday	221	356.7×10^6	1355	430	215	665	359,100	99.9
Tuesday	226	15.0×10^6	1355	--	620	990	504,900	96.6
Thursday	228	7.5×10^6	100	315	1050	490	254,800	96.6
Tuesday	233	12.5×10^6	50	185	25	85	43,400	99.7
Thursday	235	33.3×10^6	0	165	10	60	32,400	99.9
	Averages	146.8×10^6	605	380	225	420	214,200	99.85

* Deionized water (1.25 cm/6 hr, 5 days/week) was applied to model for eluation.

** Each application represents 30 g wet wt (3 g dry wt) manure slurry (except for Julian days 221 and 225, when twice this quantity was added) added with 500 ml deionized water.

† Each value represents three replicate determinations.

Table 4

Bacterial Isolates Other than E. coli Giving Blue Colony Color on M-FC Membrane Filter Test Plates at Low Colony Counts*

Culture Number	2	40	42†	105,106†	118	124†	135
Colony Color on M-FC Medium	Blue	Blue With Pink Center	Blue With Yellow Center	Blue	Dark Blue	Blue With Clear Margin	Light Blue
Colony Morphology and Consistency	Flat With Cracked Surface	Flat, Irregular	Flat, Irregular	Raised, Circular	Flat, Circular, Large Diam.	Raised, Circular	Flat, Circular, Diffused Margin
Collection Site	Utica Influent**	Utica Influent**	Utica Influent**	Model 1 Effluent	Model 1 Effluent	Model 3 Effluent	Utica Effluent
Indole	+	-	-	-	+	-	-
Voges-Proskauer	-	-	-	+	+	+	+
Citrate	+	-	+	+	+	+	+
ONPG	+	+	+	+	+	+	+
Hydrogen Sulfide	-	-	-	-	-	-	-
Urease	-	-	+	+	-	+	-
Oxidase	-	-	-	-	+	-	-
Gelatin Liquification	-	-	-	-	+	-	+
Lysine Decarboxylase	+	+	+	+	-	+	-
Arginine Dihydrolase	-	-	-	-	+	-	+
Ornithine Decarboxylase	+	-	-	-	+	-	+
Tryptophan Deaminase	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Sucrose	+	-	+	+	+	+	+
Rhamnose	-	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Sorbitol	+	-	+	+	-	+	-
Inositol	-	-	+	+	+	+	-
Amygdalin	-	-	+	+	+	+	+
Identification	?	?	Excellent	Excellent	?	Excellent	?
Genus	Escherichia	?	Klebsiella	Klebsiella	Enterobacter	Klebsiella	Enterobacter
Species	coli		pneumoniae	pneumoniae	sp.	pneumoniae	cloacae

* Blue colony color occurs at plate counts of less than 150 colonies.
** Algal lagoon water used as influent to the overland flow treatment plots.
† Colonies only occasionally blue at low numbers, usually losing color rapidly after removal from incubator.

Table 5

Bacterial Isolates Giving Blue Colony Color on M-FC Membrane Filter Test Plates At High Colony Counts*

Culture Number	6,11	21	26	31
Colony Color on M-FC Medium	Blue	Dark Blue	Blue	Blue
Colony Morphology and Consistency	Raised, Circular, Mucoid	Raised, Circular, Mucoid	Raised, Circular, Mucoid	Raised, Circular, Mucoid
Collection Site	Utica Effluent	Utica Effluent	Utica Effluent	Utica Effluent
Indole	-	-	+	-
Voges-Proskauer	+	-	-	-
Citrate	+	+	+	+
ONPG	-	-	-	-
Hydrogen	-	-	-	-
Urease	-	-	-	-
Oxidase	-	+	-	-
Gelatin Liquifaction	+	+	-	-
Lysine Decarboxylase	-	-	-	-
Arginine Dihydrolase	-	+	-	-
Ornithine Decarboxylase	-	-	-	-
Tryptophan Deaminase	-	-	+	-
Glucose	+	-	+	+
Sucrose	-	-	+	-
Rhamnose	-	-	-	-
Melibiose	+	-	-	+
Arabinose	+	-	-	+
Mannitol	-	-	-	-
Sorbitol	-	-	-	-
Inositol	-	-	+	-
Amygdalin	-	-	-	-
Identification Genus Species	Acceptable *Acinetobacter calcoaceticus* var. *anitratus*	Very Good *Pseudomonas fluorescens*	Very Good *Providencia stuartii*	Very Good *Acinetobacter calcoaceticus* var. *anitratus*

* Blue colony color usually occurs at plate counts of greater than 150 colonies.

Table 6

Incubation of Utica Test Plot Sod at 10 and 37°C for Six Days, Followed by Enumeration of Fecal Coliform-Positive Bacteria in Surface-Applied Deionized Water After Three Hours

Experimental Units	Initial Wt of Sod, kg	Surface Area of Sod, cm^2	Surface-Applied Water, ℓ*	Incubation Temperature, °C	Fecal Coliform Count No./100 ml**	False Positive Bacteria No./100 ml
1A	9.66	960	2.0	10	1360	No problem
2A	8.62	960	2.0	10	940	No problem
3A	9.00	960	2.0	10	1020	No problem
1B	11.09	960	2.4	37	2600†	Interference
2B	6.71	960	2.5	37	2730†	Interference
3B	8.35	960	2.0	37	325†	Interference

* Water volume added was quantity required to cover sod surface by approximately 1 cm.

** Each value represents three replicate determinations.

† Positive colonies were masked by high numbers of background bacteria that gave a fecal coliform-positive blue color; fecal coliform colonies were enumerated by the characteristic crusty morphology and pinkish-centered blue color of most *E. coli* colonies.

APPENDIX A: SUPPLEMENTARY DATA FOR THE UTICA FIELD SITE AND GREENHOUSE MODELS

Table A1

Physical and Chemical Characteristics of Lagoon Wastewater, Influent Wastewater, and Effluent Runoff from the Overland Flow System near Utica, Mississippi*

Parameter mg/ℓ	Lagoon Wastewater	Influent Wastewater	Effluent Runoff			
			Plot 24**	Plot 26**	Plot 27†	Plot 28†
TKN	8.95 (1.9 - 29.0)	17.6 (1.8 - 32.8)	4.05 (0.85 - 14.0)	3.9 (<0.5 - 9.3)	3.6 (<0.5 - 13.5)	4.15 (<0.5 - 17.0)
NH_3-N	3.45 (<0.01 - 12.3)	12.8 (<0.01 - 29.0)	0.87 (<0.01 - 5.7)	0.79 (<0.01 - 5.85)	0.49 (<0.01 - 7.9)	1.05 (<0.01 - 9.15)
NO_3-N	0.16 (<0.01 - 1.65)	0.42 (<0.01 - 5.0)	1.05 (<0.01 - 7.65)	1.3 (<0.01 - 9.3)	1.2 (<0.01 - 13.6)	1.2 (<0.01 - 10.9)
T-P	6.45 (1.45 - 12.3)	9.15 (2.7 - 18.3)	5.7 (<0.1 - 11.8)	6.0 (<0.1 - 11.5)	4.75 (<0.1 - 15.8)	4.85 (<0.1 - 12.5)
O-PO_4-P	5.25 (0.97 - 11.0)	7.9 (2.35 - 15.2)	5.0 (0.39 - 11.0)	5.3 (0.34 - 11.6)	4.0 (<0.01 - 15.1)	4.25 (0.08 - 12.4)
Suspended solids	--	30 (1 - 82)	13 (3 - 63)	12 (3 - 56)	5.5 (1 - 15)	15.5 (1 - 35)
BOD_5	--	22 (6 - 56)	8.5 (3 - 32)	9 (3 - 30)	3 (1 - 5)	5 (3 - 8)

* Average values; ranges are in parentheses.
** Intermittent-treatment plots, 0.21 cm/hr for 6 hr, 5 days/week.
† Continuous-treatment plots, 0.11 cm/hr for 24 hr, 7 days/week.

Table A2

Retention Time Data for the Utica Plots Using Chloride Ion as a Tracer

Plot No.	Flow Rate cm/hr	Flow Rate hr	Season	Retention Time hr*
23	0.21	6	Summer	2.75
24	0.21	6	Winter	2.36
25	0.21	6	Summer	2.25
26	0.21	6	Winter	2.52
28	0.11	24	Winter	4.38

* Average value for three measurements.

Table A3

Physical and Chemical Characteristics of Effluents from the Greenhouse Overland Runoff Models During Initial Application of Deionized Water*

Utica Plot Model 2				Cattle Pasture Model 3			
Parameter	Measurements	Range	Mean	Parameter	Measurements	Range	Mean
TKN, mg/ℓ	9	0.91 - 9.76†	3.43	TKN, mg/ℓ	14	0.67 - 2.94	1.93
NH_3-N, mg/ℓ**	9	0.03 - 2.90†	0.75	NH_3-N, mg/ℓ	14	0.025 - 0.33	0.15
NO_3-N, mg/ℓ**	9	0.43 - 56.0	11.89	NO_3-N, mg/ℓ	14	0.46 - 6.8	1.84
T-P, mg/ℓ	9	0.62 - 5.43†	2.55	T-P, mg/ℓ	14	<0.10 - 0.46†	0.23
O-PO_4-P, mg/ℓ**	9	0.65 - 4.36†	1.39	O-PO_4-P, mg/ℓ**	14	<0.01 - 0.065	0.03
Suspended solids, g/ℓ	8	0.007 - 0.77†	0.034	Suspended solids, g/ℓ	8	0.071 - 0.170	0.119
Temperature, °C	8	22.7 - 25.3	24.1	Temperature, °C	8	22.0 - 25.9	23.5
pH	4	6.6 - 7.3	7.0	pH	4	6.9 - 7.35	7.15

* Period Apr-Jul 1979 for Model 2 and May-Jul 1979 for Model 3.
** Measured in 0.45μ filtrates.
† Initial runoff gave the highest value.

Table A4

Temperature Data for Different Downslope Distances on the Greenhouse Models

Model No.	Duration	Measurements	Average Temperature, °C*				
			Influent**	0-1.5 m	1.5-3 m	3-4.5 m	Effluent
1	Mar 79	5	25.5 (23.9-27.8)	25.8 (23.4-29.5)	26.3 (23.1-28.9)	25.9 (22.8-29.5)	26.5 (22.5-30.5)
2	May-Aug 79	8	25.9 (23.2-29.0)	24.4 (21.9-25.3)	23.8 (22.2-24.9)	23.6 (22.1-25.1)	24.1 (22.5-25.9)
2	Dec 79-Jan 80	42	24.0 (16.0-30.5)	21.0 (16.0-26.4)	20.6 (15.5-24.8)	20.5 (15.2-25.1)	21.1 (15.8-26.2)
3	Apr-Jul 79	4	25.6 (25.0-26.2)	24.7 (23.5-25.4)	24.4 (23.3-25.0)	23.7 (21.9-24.7)	23.7 (22.0-24.9)

* Ranges are given in parentheses.

** Air temperature was regulated at 29.5°C during the day except during the period Dec 79 to Jan 80, when the average was 24.3 (16.0-32.8)°C.

Table A5

Two-Step M-TEC Test for Enumerating E. coli on Membrane Filters

M-TEC Medium Composition:

Proteose Peptone No. 3	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
Sodium Chloride	7.5 g
Dipotassium Hydrogen Phosphate	3.3 g
Potassium Dihydrogen Phosphate	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Brom Cresol Purple	0.08 g
Bromophenol Red	0.08 g
Agar	15.0 g

Bring up to 1.0-ℓ volume with distilled water.
The ingredients are dissolved by stirring and sterilized by autoclaving (121°C for 15 min), and 4 ml is poured into each 47-mm plate. The pH of the medium is 7.3.

Urease Substrate Composition:

Urea	2.0 g
Phenol Red	0.01 g

Dissolve the above ingredients in 1.0 ℓ of distilled water.
Adjust pH of solution to between 4.5 and 5.2.
It should be a straw-yellow color at this pH range.

Step 1: Following filtration of appropriate sample volumes through 0.45-μm membrane filters, roll filters onto surface of solidified M-TEC medium in each plate and avoid air pockets. Incubate plates at 35°C for 2 hr followed by incubation at 44°C for 20 ± 2 hr.

Step 2: Following incubation of plates, transfer countable plate filters to pads saturated with the urease substrate solution. After 15 to 20 min, count all yellow colonies as thermotolerant *E. coli*.

Table A6

Single-Step M-TEC Test for Enumerating

E. coli on Membrane Filters

Prepare Standard M-TEC medium as shown in Table A5. After the M-TEC ingredients have been autoclaved, add the following well-mixed solution of indoxyl-β-D-glucoside to 1 ℓ M-TEC medium.

Indoxyl-β-D-glucoside	0.25 g
Ethanol	5 ml
Distilled water	5 ml

The indoxyl-β-D-glucoside is broken down to glucose and indoxyl by bacteria that possess the enzyme β-D-glucosidase. Organisms having the enzyme will form blue to green colonies on the above modified M-TEC medium. Colonies of E. coli are yellow, whereas most of the other thermotolerant, lactose-positive colonies are blue to green.

APPENDIX B: SUPPLEMENTARY FECAL COLIFORM DATA

Table B1

Daily Fecal Coliform Counts from Influent and Effluent Samples Collected at the Utica Overland Flow Treatment Plots

Date	Day	Wastewater Influent No./100 ml	Effluent (1.25 cm/6 hr)* No./100 ml		Effluent (2.5 cm/24 hr)** No./100 ml	
			Plot 24	Plot 26	Plot 27	Plot 28
4 Oct 77	Tuesday	44,700	6,730	4,770	70	350
28 Oct 77	Friday	7,600	1,130	1,070	130	130
2 Nov 77	Wednesday	6,300	--	--	--	--
16 Nov 77	Wednesday	1,670	570	2,050	35	35
21 Nov 77	Monday	4,000	3,130	2,750	--	--
13 Dec 77	Tuesday	2,830	1,720	1,680	130	350
11 Jan 78	Wednesday	1,050	850	1,060	120	35
8 Feb 78	Wednesday	1,110	170	200	0	0
24 Feb 78	Friday	100	40	50	0	0
8 Mar 78	Wednesday	210	90	160	45	15
29 Mar 78	Wednesday	9	360	1,550	0	0
6 Apr 78	Thursday	105	150	870	--	--
4 May 78	Thursday	40	4,880	480	0	0
17 May 78	Wednesday	75	210	20	20	20
26 May 78	Friday	2	25†		2†	
6 Jun 78	Tuesday	1	1,470	1,750	0	0
30 Jun 78	Friday	5		††	570	30
7 Jul 78	Friday	0	51,000†		200†	
19 Jul 78	Wednesday	12	5,470	6,670	5	9
28 Jul 78	Friday	9	930†		100†	
4 Aug 78	Friday	80	380	1,100	100	130
11 Aug 78	Friday	4,760	200	200	4	490
18 Aug 78	Friday	10	7,530	760	0	20
22 Sep 78	Friday	1,075	20	490	230	70
29 Sep 78	Friday	18,300	2,130	1,730	1,070	330

(Continued)

* Averages for Plots 24+26, run 5 days/week; 2 percent slope on the plots.

** Averages for Plots 27+28, run continuously; 2 percent slope on the plots.

† Composite of Plots 24/26 or 27/28.

†† Data excluded because of excessive background bacteria colonies on M-FC plates.

Table B1 (Concluded)

Date	Day	Wastewater Influent No./100 ml	Effluent (1.25 cm/6 hr)* No./100 ml		Effluent (2.5 cm/24 hr)** No./100 ml	
			Plot 24	Plot 26	Plot 27	Plot 28
17 Nov 78	Friday	780	1,570	2,850	150	650
30 Nov 78	Thursday	23,500	2,870	7,930	2,970	2,130
14 Dec 78	Thursday	7,750	4,930	4,670	1,380	--
5 Apr 79	Thursday	2,350	270	4,570	60	460
9 May 79	Wednesday	400	4,430	2,270	25	25
20 Jun 79	Wednesday	0	4,370	150	7	240
11 Jul 79	Wednesday	2,430	890	340	240	1,700
18 Jul 79	Wednesday	45	320	130	590	100
26 Jul 79	Thursday	3,370	420	360	230	80
2 Aug 79	Thursday	75	180	900	210	580
8 Aug 79	Wednesday	115	250	50	80	25
15 Aug 79	Wednesday	2,400	370	1,630	90	15
23 Aug 79	Thursday	9,200	660	760	10	80
30 Aug 79	Thursday	70	880	350	0	210
6 Sep 79	Thursday	120	1,300	950	110	170
13 Sep 79	Thursday	210	630	660	25	820
3 Oct 79	Wednesday	880	6,700	2,200	160	440
4 Oct 79	Thursday	1,600	1,770	1,330	20	25
10 Oct 79	Wednesday	840	100	100	0	10
16 Oct 79	Tuesday	160	45	45	0	7
17 Oct 79	Wednesday	120	--	--	--	--
18 Oct 79	Thursday	8	40	15	3	10
24 Oct 79	Wednesday	5,470	380	850	7	25

Table B2

Response of Greenhouse Overland Runoff Model 1 to the Application of Deionized Water Containing a Morphologically Distinct E. coli, and Effect of Sample Storage on E. coli Recovery by the M-FC Membrane Filter Test

Day	Julian Date	Influent E. coli No./100 ml	Effluent E. coli, No./100 ml					
			Initial	1 hr	1.5 hr	2.5 hr	3.5 hr	Composite
Tuesday	030	1,460	350	--	--	340	--	260
Wednesday	031	700	--	--	--	60	--	85
Thursday	032	1,750	275	200	--	120	--	135
Monday	036	35,000	11,800	--	--	--	--	11,800
Tuesday	037	16,100	3,250	--	--	2,950	--	2,350
Wednesday	038*	4,830	730	--	--	500	--	630
Thursday	039	1,460	225	--	--	340	--	360
Friday	040	3,780	1,150	--	--	770	--	1,120
Monday	043*	4,530	†	†	†	†	†	†
Tuesday	044	3,850	1,655	--	--	--	--	--
Wednesday	045*	1,900	990	--	--	170	--	--
Thursday	046*	5,830	2,950	--	--	480	--	--
Friday	047	2,650	730	--	--	390	--	630
Tuesday	051*	920	†	†	†	†	†	†
Wednesday	052*	770	910	--	--	430	--	--
Thursday	053*	1,330	470	--	--	290	--	--
Friday	054	12,050	810	--	--	--	--	2,260
Wednesday	059	1,385	--	--	--	--	--	125
	059*	265	--	--	--	--	--	--
Thursday	060	0	0	7	--	--	--	--
Friday	061	0	0	0	--	--	--	--
Monday	064	965	65	--	--	--	--	--
Wednesday	066	1,317,000	1,365	--	--	--	--	--
	066*	1,183,000	2,155	††	††	††	††	††
	066**	--	1,955	230,000	170,000	145,000	--	--
Thursday	067	18,200	12,000	--	--	--	--	--
	067*	16,200	10,600	††	13,600	6,600	4,630	--
Friday	068	82,700	--	12,300	--	--	--	--
	068*	71,900	8,070	12,530	9,830	5,670	5,600	--
Saturday	069	0	2,300	11,000	10,030	--	--	--
Tuesday	072	0	320	330	--	--	--	--
	072*	0	653	223	143	90	--	--
Wednesday	073	0	--	190	107	55	--	--
Thursday	074*	0	95	60	185	--	--	--
Tuesday	079	12,250	50	340	720	--	--	--
	079*	10,500	25	775	655	725	405	--
Wednesday	080*	0	25	--	--	--	--	--

* The fecal coliform test was run on samples stored for approximately 18 hr at 4°C.
** The fecal coliform test was run on samples stored for approximately 42 hr at 4°C.
† No runoff occurred.
†† Too numerous to count.

Table B3

Morphological and Biochemical Variability of Escherichia coli, Isolated from Overland Runoff with the M-FC Membrane Filter Test

Culture Number	4	5	41	43	44	51	52	54	65,66	121	139	140	141
Colony Color On M-FC Medium	Blue with pink center	Blue with pink center	Blue with pink center	Blue	Blue with clear margin*	Blue with pink center	Blue	Blue	Blue	Blue with pink center	Blue with pink center	Blue with clear margin	Grey blue
Colony Morphology And Consistency	Flat, irregular	Flat, irregular	Flat, irregular	Flat, cracked surface	Raised circular, mucoid	Flat, circular	Flat, cracked surface	Flat, irregular	Flat, irregular	Flat, irregular	Flat, irregular	Raised, circular, mucoid	Flat, irregular mucoid
Collection Site	Utica Influent**	ATCC 11303	Utica Effluent	Utica Influent**	Utica Effluent	Utica Influent**	Utica Influent**	Utica Effluent	Utica Effluent	Model 2† Effluent	Model 2 Effluent	Model 3†† Effluent	Model 3 Effluent
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrogen Sulfide	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin Liquification	-	-	-	-	-	-	-	-	-	-	-	-	-
Lysine Decarboxylase	+	+	-	-	-	-	+	+	+	+	-	+	+
Arginine Dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine Decarboxylase	-	-	-	-	-	+	-	-	+	-	+	+	+
Tryptophan Deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	-	-	-	+	-	-	-	-	+	+	+	-	-
Rhamnose	+	+	+	+	+	+	-	+	+	+	+	-	-
Melibiose	+	+	-	+	-	+	+	+	+	+	+	-	-
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	-	+	-	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
Amygdalin	-	-	-	-	-	-	-	-	-	-	-	-	-
Identification	Excellent	Excellent	Very Good	Very Good	Very Good	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

* Lacking characteristic granular structure in colony matrix.
** Algal lagoon wastewater, used as influent to the overland flow treatment plots.
† Greenhouse model; a tracer (Strep. - resistant) E. coli was added to the deionized water influent; sod was obtained from within a field plot at Utica.
†† Greenhouse model; cattle manure was eluted with deionized water; sod was obtained from a cattle pasture at Utica.

Table B4

Morphological and Biochemical Variability of Klebsiella Pneumoniae, Isolated from Overland Runoff with the M-FC Membrane Filter Test

Culture Number	3	16	17	19	24	27	42	105,106	122	123	124
Colony Color On M-FC Medium	Brown center, clear margin	Pale green	Pink	Brown	Clear	Clear	Blue with yellow center	Blue	Grey blue	Pink	Blue with clear margin
Colony Morphology And Consistency	Flat, irregular	Raised, circular	Raised, circular	Raised, circular	Flat, irregular	Flat, cracked surface*	Flat, irregular*	Raised, circular	Flat, circular	Raised, circular	Raised circular
Collection Site	Utica Effluent	Utica Effluent	Utica Influent**	Utica Effluent	Utica Effluent	Utica Effluent	Utica Influent**	Model 1† Effluent	Model 2†† Effluent	Model 3‡ Effluent	Model 3 Effluent
Indole	-	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer	-	-	-	-	-	+	-	+	+	+	+
Citrate	+	+	+	+	-	+	+	+	+	+	+
ONPG	+	+	+	+	+	+	+	+	+	+	+
Hydrogen Sulfide	-	-	-	-	-	-	-	-	-	-	-
Urease	+	+	+	+	-	-	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-
Gelatin Liquification	-	-	-	-	-	-	-	-	-	-	-
Lysine Decarboxylase	+	+	+	+	+	+	+	+	+	+	+
Arginine Dihydrolase	-	-	-	-	-	-	-	-	-	-	-
Ornithine Decarboxylase	-	-	-	-	-	-	-	-	-	-	-
Tryptophan Deaminase	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	+	+
Amygdalin	+	+	+	+	+	+	+	+	+	+	+
Identification	Excellent	Excellent	Excellent	Excellent	Very Good	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

* Lacking the characteristic mucoid colony consistency.
** Algal lagoon wastewater used as influent to the overland runoff treatment plots.
† Greenhouse model; a morphologically distinct E. coli was added to the deionized water influent; sod was obtained outside of field test plots at Utica.
†† Greenhouse model; a tracer (Strep.-resistant) E. coli was added to the deionized water influent; sod was obtained from within a field test plot at Utica.
‡ Greenhouse model; cattle manure was eluted with deionized water; sod was obtained from a cattle pasture at Utica.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Hoeppel, Ronald E

Fate and enumeration problems of fecal coliform bacteria in runoff waters from terrestrial ecosystems / by Ronald E. Hoeppel, R. Glenn Rhett, C. Richard Lee. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1980.

97, [19] p. : ill. ; 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; EL-80-9)

Prepared for Assistant Secretary of the Army (R&D), Department of the Army, Washington, D. C., under Project No. 4A161101A91D, Task 02.

References: p. 88-97.

1. Bacteria. 2. Coliforms. 3. Effluents. 4. Field tests. 5. Model tests. 6. Overland flow. 7. Surface runoff. 8. Waste water disposal. 9. Waste water treatment. I. Rhett, R. Glenn, joint author. II. Charles Richard Lee, joint author. III. United States. Assistant Secretary of the Army (Research and Development). IV. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Technical report ; EL-80-9.

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1 September

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Errata Sheet

No. 1

FATE AND ENUMERATION PROBLEMS OF FECAL COLIFORM BACTERIA IN RUNOFF WATERS FROM TERRESTRIAL ECOSYSTEMS

Technical Report-EL 80-9

September 1980

1. Page 85, paragraph 141, line 15 should be changed to read:

   tion of 0.1-0.2 cm per hour during a 12- to 18-hr period each day (7 d

2. Page 86, paragraph 147, second sentence should read:

   The recommended rate for best treatment is daily application at 0.1-0.2 cm/hr for 12-24 hr.