A STUDY OF DECAY OF FAECAL COLIFORM
IN
AQUATIC ENVIRONMENT

BY

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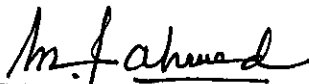
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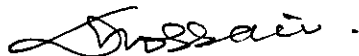
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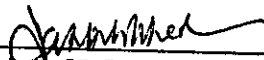BUET, Dhaka 1000

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I hereby certify that the research work reported in this thesis has been performed by me and this work has not been submitted elsewhere for any other purpose (except for publication).

September, 1996

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ABSTRACT

Prediction of microbial water quality in surface water bodies is essential to provide an indication of health risks posed by faecal contamination. Due to the great difficulties involved in the detection and enumeration of individual pathogens, indicator organisms are usually used to assess the microbial water quality. Faecal coliform bacteria is widely used as an indicator of microbial water quality in water supply engineering. Faecal coliform enter water sources mainly through discharge of sewage and waste water or urban and rural runoff. Once in water, various factors act to enhance or inhibit their growth. For accurate prediction of microbiological water quality a thorough understanding of the effect of various factors on the survival of faecal coliform is essential.

This study was devoted to acquire a better understanding of the decay mechanism of faecal coliform in natural water environment. The decay of faecal coliform was observed under various laboratory conditions and also in actual lake and stream environment. The effect of temperature, pH and nutrient deficiency on the decay of faecal coliform was observed in the laboratory. Decay rate of faecal coliform in Dhanmondi lake water and in the Buriganga river water was determined both in the laboratory and in the actual aquatic environment.

Temperature was found to have a major impact on the survival of faecal coliform in aquatic environment. At very low temperatures the bacterial population remained nearly constant. But as the temperature increased above 20°C the decay rate started increasing rapidly. At about 50°C faecal coliform cannot survive for more than 6 hours. pH of the aquatic environment also significantly affects the decay rate of faecal coliform. However, near neutral condition, the variation of decay rate with change in pH level is very small. But as the deviation becomes larger the change in decay rate also becomes greater. Survival is more strongly affected by acidic environment than by alkaline environment. Nutrients usually retard the decay of faecal coliform. At a very high nutrient concentration, a small after-growth was observed. However, the nutrient

concentration and pH of the natural water bodies rarely reach those levels to significantly affect bacterial decay.

Decay rate of faecal coliform in both river water and lake water under laboratory condition is significantly higher than that in sterilised distilled water. Presence of toxic substances and trace metals are responsible for this. Decay rate in actual river environment was 10% higher than that in the laboratory. It indicates that the combined effect of sedimentation, resuspension, biological extraction and irradiance in the ultraviolet range is not very significant on the loss rate of faecal coliform in the actual stream environment.

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α	Proportionality constant for irradiance mediated death rate of faecal coliform
I_0	Incident solar radiation, $Cal/cm^2.min$
K_1	Decay rate of faecal coliform, day^{-1}
K_{20}	Decay rate of faecal coliform at 20^oC
K_B	Decay rate at the surface of water, day^{-1}
K_{BO}	Depth-averaged decay rate due to sunlight, day^{-1}
K_d	Death rate as a function of temperature, predation, salinity etc., day^{-1}
K_c	Light extinction coefficient, m^{-1}
K_g	After growth rate, day^{-1}
K_i	Rate coefficient for death of faecal coliform as mediated by irradiance
K_l	Overall loss rate in the lake environment, day^{-1}
K_r	Overall first order loss rate of faecal coliform in lake, day^{-1}
K_s	Loss rate due to settling, day^{-1}
v_s	Net loss rate due to settling and resuspension, LT^{-1}

CHAPTER 1
INTRODUCTION
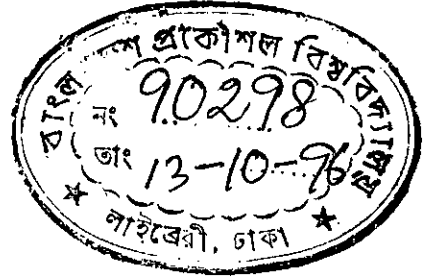
1.1 GENERAL

Water is essential for the survival of both animal and plant. It is difficult to imagine any clean and sanitary environment without water. Invariably, the progress of sanitation throughout the world has been closely associated with the availability of water. The larger the quantity of and the better the quality of the water, the more rapid and extensive has been the advance of public health.

While man has always recognised the importance of water for his internal body needs, his recognition of its importance to health is a more recent development, dating back only a century or so. Since that time, much has been learned about the role of inadequate and contaminated water supplies in the spread of water-borne diseases. Among the first diseases recognised to be water-borne were cholera and typhoid fever. Later, dysentery, gastro-enteritis and other diarrhoeal diseases were added to the list. More recently water has also been shown to play an important role in the spread of certain viral diseases such as infectious hepatitis (jaundice).

Water is involved in the spread of communicable diseases in essentially two ways. The first is the well known direct ingestion of infectious agent when drinking contaminated water. The second is due to a lack of sufficient water for personal hygiene purposes. Inadequate quantities of water for the maintenance of personal hygiene and environmental sanitation have been shown to be major contributing factors in the spread of epidemic diseases. Levine, et al. found that tubewell water drinkers did not have lower cholera

infection rates and attributed this to regular use of contaminated surface water sources for all other purposes (Skoda et al., 1977).

Approximately 80 percent of all sicknesses and diseases can be attributed to inadequate water supply and sanitation. For example, diarrhoeal disease kill six million children in developing countries every year and affects upto 18 million people. People with water-borne diseases occupy about half of all the hospital beds in the world. World Health Organisation (WHO) estimates that about 25,000 people die from water-related diseases every day (Rahim et al., 1985).

On a global basis approximately three out of five persons in developing countries have no access to safe drinking water. Urban areas are better served, 75 percent of the population have some form of water supply through house connections or outdoor stand pipes. In the rural areas only 29 percent have access to safe drinking water. (Bilqis et al, 1988)

Grant (UNICEF, 1989) mentioned that 250,000 children of Bangladesh die every year from diarrhoeal dehydration. UNICEF has also reported that one third of child deaths are due to diarrhoeal diseases and only 30 percent of urban population has access to reasonable water supply.

1.2 STATEMENT OF THE PROBLEM

Bangladesh, the 144,000 sq. km delta in the Bay of Bengal, is inhabited by 130 million people who are mostly illiterate and are among the lowest per capitata income generating group of the world. Rivers, ponds, canals, ditches, etc. have been the sources of drinking water for ages. In comparison to

existing tubewells, often these surface sources have been more convenient, easily accessible and socio-economically preferable to the majority of the population. But the practices of indiscriminate defecation and disposal of raw sewage into these surface water bodies have created an environment which is heavily polluted and is of major concern because it has every potential to serve as reservoir or carrier of pathogens of water-related diseases (Bilqis et al. 1988).

The water sources in and around the densely populated cities in Bangladesh are being increasingly polluted. It has been found that average coliform counts per 100 ml of river water at Chadnighat (Buriganga river) increased from about 1500 in 1968 (Rahman et al., 1994) to more than 40,000 in 1996. Bacteriologically the rivers Buriganga, Sitalakhya, Balu around Dhaka and Karnaphully in Chittagong are 3 to 7 times more polluted than the rivers Padma and Jamuna flowing by small cities and open areas. Surface waters in ponds and lakes also often show high coliform counts. Many surface waters receiving sewage and human waste are characterised by extremely high faecal coliform counts (Ahmed, 1988).

Table 1.1 Bacteriological quality of water of some rivers in Bangladesh (Ahmed, 1988)

River	Maximum(coliform count/100 ml)	Minimum (coliform count/100 ml)	Average (coliform count/100 ml)
Buriganga at Chadnighat	12600	1000	7050
Sitalakhaya at Demra	12000	1000	3683
Karnafuly at Chittagong	2500	500	1446
Jamuna at Bahadurabad	1200	150	1050
Padma at Paksey	3800	750	1559

The problem of microbial pollution of surface water sources is increasing with the increase in population, urbanisation, and indiscriminate discharge of waste waters. Surface waters are used by millions of people in Bangladesh for various purposes. The risk involved in the use of such water sources is dependent on the concentration of pathogens which is indicated by the concentration of faecal coliform.

1.3 SIGNIFICANCE OF THE STUDY

The three elements of the life support system-- water, food, air-- are essential for human survival, but they are also potential vehicles for the spread of disease causing micro-organism as well as toxic chemicals. One of the major

concerns of the environmental technologist must be the protection of the public health by preventing contamination of the life support system with harmful organisms and chemicals.

In our country most of the domestic and municipal sewage is discharged with little or no treatment. These wastes contain a large quantity of pathogens especially of intestinal origin that cause waterborne diseases. Most of these wastes find their way into the natural streams through surface runoff and municipal sewer overflow. So the only source of purification is the assimilation capacity of the streams and hence the microbial self purification is a vital factor. Bacteriological standards, based on faecal coliform group, is currently accepted as the indicator of microbial quality of surface waters. The decay rate of faecal coliform governs the permissible limit of microbial loading into the stream and dictate the degree of treatment required prior to discharge into the natural stream. Also the utility of faecal coliform bacteria as an indicator of presence of pathogenic bacteria depends to a degree on the survival of faecal coliform bacteria under different environmental conditions. Unfortunately, till now, there had been very little study on the decay of faecal coliform in a tropical country like Bangladesh.

1.4 OBJECTIVE OF RESEARCH

This study was undertaken to provide a better understanding of the complex processes that govern the microbial self purification in aquatic environment. The specific objectives of the proposed research study are as follows:

(i) To study the decay of faecal coliform under different conditions to have a better understanding of bacterial decay kinetics;

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(ii) To study the influence of the most variable water quality parameters such as temperature, pH, nutrients, on the decay of faecal coliform;

(iii) To determine the decay rate of faecal coliform in actual lake and river environment and to predict the microbial self purification capacity of these water bodies;

(iv) To assess the applicability of faecal coliform bacteria as indicator of microbial water quality under different environmental conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 PRIMARY WATER BORNE PATHOGENS

A variety of different micro-organisms are found in untreated water. Only a small fraction of these micro-organisms pose health hazards to human and are generally known as pathogens. Pathogens are not native to aquatic systems and usually require an animal host for growth and reproduction. They can however be transported by natural water systems, thus becoming a temporary member of the aquatic community. Many species of pathogens are able to survive in water and maintain their infectious capabilities for significant periods of time. Micro-organisms posing health hazard includes species of bacteria, virus and protozoa.

2.1.1 Bacteria of Public Health Significance

Bacteria are unicellular procaryotic organisms and are the lowest form of life capable of synthesising protoplasm from the surrounding environment. Bacteria of interest in drinking water are *Salmonella, Shigella, Yersinia enterocolitica, campylobacter jejuni, Leginella, entero pathogenic E. coli, Vibrio cholerae, Mycobacterium* (AWWA, 1990).

Salmonella. Over 2200 known serotypes of *Salmonella* exist, all of which are pathogenic to humans. Most of them causes gastro-enteritis; however , *S. typhi* and *S. paratyphi* cause typhoid and paratyphoid respectively. These two species infect only humans; the others are carried by both humans and

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animals. Salmonella are transmitted through water or food processed with contaminated water.

Shigella. Four main subgroups exist in this genus, *S. sonnei, S. flexneri, S. boydii,* and *S. dysenteriae.* They infect humans and primates and cause basillary dysentery. *S. sonnei* causes the bulk of water borne infections although all four subgroups have been isolated during different disease outbreaks. Waterborne shigellosis is most often the result of contamination from one identifiable source, such as inadequately maintained and/or monitored water systems. These organisms are usually transmitted through contaminated food, polluted water, and person-to-person contact.

Yersinia enterocolitica. This organism can cause acute gastro-enteritis and is carried by humans and a variety of animals. *Yersinia enterocolitica* can grow at temperatures as low as 4^0 C and has been isolated in untreated surface water more frequently during colder months. The disease symptoms accompanied with an infection of *Yersinia enterocolitica* include diarrhoea, fever, vomiting, anorexia, acute abdominal pain, abscesses etc.

Campylobacter jejuni. This organism can infect humans and a variety of animals. In humans it causes acute gastro-enteritis for a period of about one week.

Legionella. Twenty six species of *Legionella* have been identified. Seven are etiologic agents of Legionnaires disease; however, *Legionella pneumphila* is the primary causative agent. *Legionella* organisms can cause effects similar to acute pneumonia or a milder nonpneumonic illness designated pontiac fever. The former typically is found in immunosuppressed individuals, or those who are susceptible due to age, smoking or underlying illness.

Enteropathogenic *E. coli*. Approximately 11 of the more than 140 existing serotypes of *E. coli* cause gastro-enteritis in humans. *Enteropathogenic E. coli* is a prime cause of diarrhoea in infants. These organisms are usually transmitted through sewage contaminated food or water and via person to person contact.

Vibrio cholerae. This virulent produces acute intestinal disease with diarrhoea, vomiting, suppression of urine, dehydration, reduced blood pressure, subnormal temperature and complete collapse. Death may occur within few hours unless medical care is given. *Vibrio cholerae* is usually transmitted through contaminated water and person to person contact.

Mycobacteria. *Mycobacteria tuberculosis* causes tuberculosis in humans. It is typically transmitted through person to person contact; however sewage contaminated water is a potential pathway. Species of non tubercular mycobacteria, *M. Kansasii* and *M. avium-intracellulare*, can cause pulmonary and other diseases in individuals. Those identified as being of highest risk are the very young, the elderly and persons with pre-existing health conditions. Some evidence exists linking drinking water to disease caused by micobacteria. Potable water can, however support their growth and transmit them through the distribution system. In addition, waterborne *M. avium-intracellulare* has been epidemiologically linked to infections in hospital patients.

Leptospira. Common human isolates includes *L. pomona, L. auumnails, L. austrails.* Leptospirosis, i.e., acute infections of kidney, liver and central nervous system is the disease associated with these organisms. It is

transmitted through skin abrasions or mucous membranes to blood stream; may come from contact with animal carriers and/or polluted waters.

Pasturella. *P. Tularensis* is responsible for Tularemia, which causes chills and fever, with an ulcer at site of infection and swollen nodes. Possible sources of transmission includes handling of infected animals, anthropod bite and drinking contaminated water.

2.1.2 Viruses of Public Health Significance

Viruses are the smallest biological structures known to contain all the genetic information necessary for their own reproduction. They require a host to live. They do not grow on artificial media- require living cells within which they are reproduced. Electron microscope is required to see viruses. Multiplication takes place by replication.

Enteric viruses infect the GI tract of humans and, in some cases, animals and are excreted in the faeces of infected people or animals. Over 100 types of enteric viruses exist. Enteric viruses of particular interest in drinking water are hepatitis A, Norwalk-type viruses, rotaviruses, adenoviruses, enteroviruses and, reoviruses.

Hepatitis A. Although all the enteric viruses are potentially transmitted by drinking water, evidence of this route of infection is strongest for hepatitis A (HAV). HAV causes infectious hepatitis, an illness characterised by inflammation and necrosis of the liver. Symptoms include fever, weakness, nausea, vomiting and diarrhoea. Jaundice may also occur.

Norwalk-type viruses. The Norwalk-type viruses cause acute epidemic gastro-enteritis. At least three serologically distinct viruses exist in this group. Like HAV, these viruses have not been cultured in the laboratory; however immunochemical methods exist for detecting their antigens and antibodies. Evidence of waterborne disease outbreak attributed to Norwalk-type viruses is based on serological evidence from individuals who are ill.

Rotaviruses. Rotaviruses cause acute gastro-enteritis, primarily in children. In developing countries, rotavirus infections are a major cause of infant mortality.

Adenoviruses, enteroviruses, and reoviruses. Viruses in each of these groups can infect both the enteric and upper respiratory tract. Adenoviruses have been detected in waste water and contaminated surface water, but not to date in drinking water. Entero- and reoviruses have been detected in waste-water, natural water and finished drinking water. No outbreaks of illness from drinking water contaminated with these viruses have been documented; therefore their significance as disease causing agents in drinking water is uncertain. Drinking water has been implicated as the route of transmission for the poliomyelitis virus (an enterovirus), but the epidemiological evidence is not conclusive.

2.1.3 Protozoans

Protozoans are unicellular, colourless, generally motile organisms that lack a cell wall. Various groups of protozoans exist, including amoebas, flagellated protozoans, ciliates, and sporozoans. Several protozoans are pathogenic to

humans. Pathogenic protozoans of interest in drinking water are *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium*, and *Naegleria fowleri*.

Giardia lamblia. This flagellated protozoan can exist as a trophozoite, 9 to 21μm long, or as an ovoid cyst, approximately 10 μm long and 6 μm wide. When ingested, *Giardia* can cause giardiasis, a GI disease manifested by diarrhoea, fatigue and cramp. Symptoms may last any where from a few days to months. Water can be a major vehicle for transmission of giardiasis, although person-to-person contact and other factors may be more important. Both humans and animals, particularly beavers and muskrats, are reservoirs for *Giardia*.

Entamoeba histolytica. This protozoa exists either as a fragile trophozoite, 15 to 25 μm long, or as more hardy cyst, 10 to 15 μm in diameter (slightly higher than *Giardia*). When ingested, *Entamoeba* can cause amoebic dysentery, with symptoms ranging from acute diarrhoea and fever to mild gastro-enteritis. Occasionally they can invade the bloodstream, reach other organs (typically the liver), and cause amoebic abscesses.

Cryptosporidium. A variety of species of the protozoan *Cryptosporidium* have been identified. Two are associated with infections in mammals, *C. muris* and *C. paryum*. Isolates from animals have been shown to initiate infections in human. The primary symptom of cryptosporidiosis is acute diarrhoea. Other symptoms may include abdominal pain, vomiting, and low grade fever. Sources of infection include pets and farm animals, person-to-person contacts, and contaminated drinking water. The infectious dose have not been well defined.

Naegleria fowleri. This free-living amoeba is found in soil, water, and decaying vegetation. The trophozoite measures 8 to 15 μm. Exposure is typically from swimming in freshwater lakes having high concentrations of the organism. The route of infection is via the nasopharynx, through the olfactory epithelium, and up the olfactory nerve plexus to the brain. Primary amoebic encephalitis results with symptoms of headache, high fever, and somnolence. Death usually occurs on the fifth or sixth day. All disease incidents have been associated with swimming in natural water; treated drinking water supplies are not a suspected route of transmission.

2.2 INDICATOR ORGANISMS

Testing a water sample for pathogenic bacteria might at first glance be considered a feasible method for determining its bacteriological quality. However on closer examination, this technique has a number of shortcomings that precludes its application.

- Pathogens are likely to gain entrance sporadically, and they do not survive for very long period of time consequently they could be missed in a sample submitted to the laboratory.

- Although it is possible to detect the presence of various pathogens in water, the isolation and identification of many of these are often extremely complicated and seldom quantitative.

- Analysis of water for all known pathogens would be a very time consuming and expensive proposition.

Tests for specific pathogens are usually made only when there is a reason to suspect that those particular organisms are present. At other times, the microbiological quality of water is checked using indicator organisms.

An indicator organism is one whose presence presumes that contamination has occurred and suggests the nature and extent of the contaminant(s).

2.2.1 Choice of Indicator Organisms

It is known that most pathogens are likely to be transmitted via the water route are shed in human and/or animal faeces. Hence, an indicator organism should be a micro-organism whose presence is evidence of faecal contamination of warm blooded animals. Indicators may be accompanied by pathogens, but typically do not cause disease themselves.

The ideal indicator organism should have the following characteristics:

- be applicable to all waters
- always be present when pathogens are present
- always be absent where pathogens are absent
- numbers should correlate the degree of pollution
- be present in greater number than pathogens
- there should be no after growth or regrowth in water
- there should be greater or equal survival time than pathogens
- be easily and quickly detected by simple laboratory tests
- should have constant biochemical and identifying characteristics
- harmless to humans

A number of microorganisms have been evaluated as indicators, including total coliforms, faecal coliforms, *E. coli*, faecal streptococci, Pseudomonas aeruginosa, enterococci, and HPC. Yeasts have also recently been proposed as effective indicators. However total coliforms and the faecal coliforms have remained as the indicator of choice for decades, mainly because no other indicator has been proven to be more comprehensive than these two.

2.2.2 Total Coliform

The coliform group of organisms, referred to as total coliform, is defined in water bacteriology as all the aerobic and facultative, gram-negative, rod-shaped bacteria that ferment lactose with gas formation within 48 h at 35° C. This is an operational rather than a taxonomic definition and encompasses a variety of organisms, mostly of intestinal origin. The definition includes *E. coli*, the most numerous facultative bacterium in the faeces of warm-blooded animals, plus species belonging to the genera *Enterobacter*, *Klebsiella*, and *Citrobacter*. These latter organisms are present in wastewater but can be derived from other environmental sources, such as soil and plant materials.

No organism fulfils all the criteria for an indicator organism, but the coliform bacteria fulfil most. Drawbacks to the use of total coliform as an indicator include their regrowth in water, thus becoming part of the natural aquatic flora. Their detection than becomes false positive. False positives can also occur when the bacteria *Aeromonas*, which can biochemically mimic the coliform group, is present in the sample. On the other hand, false negatives can occur when coliforms are present along with high populations of HPC bacteria. The latter organisms may act to suppress coliform activity. Finally a number of pathogens have been shown to survive longer in natural water

and/or through various treatment processes than coliforms. This is particularly true for protozoans and viral pathogens. Despite these drawbacks, the total coliform measure remains the most useful indicator of drinking water microbial quality.

2.2.3 Faecal Coliform

Faecal coliforms provide stronger evidence of the possible presence of faecal pathogens than do total coliforms. Faecal coliforms are a subgroup of total coliforms, distinguished in the laboratory through the elevated temperature tests (i.e., $44.5\pm0.2^{\circ}C$). This more stringent conditions eliminate most of the non-faecal component while still permitting the faecal component to survive. Unfortunately there are major non-faecal sources of faecal coliform, most commonly the *klebsiella* species (Pelczar et al., 1982). Pulpmill waste water provides a frequent example.

2.2.4 Faecal Streptococci

The other group of non-pathogenic organisms proposed as indicators of faecal contamination are the faecal streptococci. The varieties considered strictly as faecal in origin are: *S. faceless, S. faecalis* var. *liquefaciens, S. faecalis* var. *zymogenes, S. durans, S. faecium, S. bovis* and *S. equinus*. Large numbers of these nonpathogens occur normally in faeces, their abundance being on the same order of magnitude as that of coliforms. It has been thought that the faecal streptococcus group occurs only in the faeces of humans and other warm-blooded animals and therefore constitutes a more specific test for faecal contamination than the coliform group. However, recent studies indicate that

streptococci similar to the faecal streptococci may also be found on certain plants, plant products, and in waste from food processing plants. The streptococcus group, although not replacing the coliform group as the standard, is considered to be a confirmation that coliform organisms found in water samples are of faecal origin.

The streptococci are more resistant than the coliforms to chlorine and survive longer in sea and brackish waters; hence this group is considered to be a more sensitive test for swimming pools and bathing waters. Moreover, Since the streptococci die out rapidly in soil whereas the coliforms may persist for long periods, the streptococcus group is considered to be a more sensitive indicator of recent faecal contamination.

2.2.5 Maximum Allowable Limits of Bacteriological Contamination

To date there are no universally established limits on allowable numbers of indicator organisms in different waters other than those that apply to the Total and Faecal Coliform groups. Drinking water standards generally specify that a water is safe provided that testing in a specified manner does not reveal more than an average of one coliform organism per 100 ml. This criterion is supported by the following arguments. The number of pathogenic bacteria, such as, *Salmonella typhosa*, in domestic water is generally less than one per million coliforms, and the average density of enteric viruses has been measured as a virus to coliform ratio of 1:100000. The die-off rate of pathogenic bacteria is greater than the death rate of coliforms outside the intestinal tract of animals; thus exposure to treatment and residence in surface waters reduces the number of pathogens relative to coliforms. Based on these premises, water that meets a standard of less than one coliform per 100 ml is,

statically speaking safe for human consumption because of the improbability of ingesting any pathogens. Strictly speaking, this coliform standard as established by the Environmental Protection Agency applies only to processed water where treatment includes chlorination.

Extension of coliform criteria to water quality for purposes other than drinking is poorly defined. Since these bacteria can originate from warm-blooded animals, soil, and cold-blooded animals in addition to faeces of man, presence of coliforms in surface waters indicates any or a combination of three sources: wastes of man, farm animals, or soil erosion. However, numerical coliform criteria for body-contact water use and general recreation have been established by most of the regulating agencies. The upper limit is 200 faecal coliforms per 100 ml or 2000 total coliforms per 100 ml.

Recommended limits of total and faecal colifrom for different types of water-use are given in the Table 2.1 (Millipore Corporation, U.S.A.).

Recently Department of Environment (DOE), Bangladesh has also set environmental quality standards (EQS, 1992). This standard has classified water into 7 catagories according to use. The standards for total coliform and faecal coliform is shown in Table 2.2.

Table 2.1 Recommended limits of total and faecal coliform

Type of	Total Coliform per 100 ml		Faecal Coliform per 100 ml	
Water	Desirable	Permissible	Desirable	Permissible
Potable and well water	0	1	0	0
Primary Contact Water (Swimming)	<1000	<2400	<200	<1000
Secondary Contact Water (Boating, Fishing)	<5000	<1000	<1000	<5000
Treated Sewage Effluent	Coliform levels should not exceed those of water receiving the discharge			

Table 2.2 Bangladesh standard for microbial water quality (DOE, 1992).

Type of water use	Total coliform (#/100 ml)	Faecal coliform (#/100 ml)
Drinking	2	0
Recreational	200	N.S.
Fishing	5000	N.S.
Industrial	N.S.	N.S.
Irrigation	1000	10
Livestock	100	N.S.
Coastal	1000	N.S.

N.S. No Standard is yet set

The standard limits of microbial loading for effluents set by DOE is listed in Table 2.3.

Table 2.3 Standard limits of total and faecal coliform in effluents (DOE, 1992)

Type of effluents	Discharge into	Total coliform (# / 100ml)	Faecal Coliform (# / 100 ml)
Sewage effluent		1000	N.S.
industrial effluent	Inland Surface Water	10000	100
	Public Sewer	10000	100
	Land	10000	100

N.S. Standard is not yet set

2.3 ORGANISM DECAY RATE

The survival, fate, and distribution of bacteria and other organisms in natural waters depend on the particular type of water body (i.e., stream, estuary, lake) and associated phenomena that influence the growth, death and other losses of the organisms. The factors that influence the kinetic behaviour of the communicable disease organisms and the indicator organisms can be conveniently classified into three categories: physical, physiochemical and biochemical-biological.

Physical factors:

- Temperature

- Salinity
- Sunlight
- sedimentation
- Adsorption
- flocculation
- Resuspension of particulates with associated sorbed organisms

Physiochemical factors:

- pH
- Osmotic effects
- Chemical toxicity
- Redox potential

Biochemical-biological factors:

- Predation
- Nutrient deficiencies
- Contact opportunity and biological extraction
- Competitive life

These factors are described in the following paragraphs.

2.3.1 Temperature

Of the physical factors affecting microbial growth in any environment, one of the most influential in the selection of species is temperature. Microorganisms possesses no means of controlling internal temperature, and the temperature within the cell is therefore determined by the external temperature. Each

organism is able to grow only within a specific range of temperatures. Fortunately, many human pathogens, having adapted to an environment ---- the human body ---- where the temperature is constant, are stenothermal and incapable of growing at temperatures more than a few degrees lower or higher than $37^{\circ}C$. This prevents their proliferation in surface waters or in such waste treatment facilities as oxidation ponds, activated sludge tanks, and trickling filters.

Temperature affects many of the factors that influences the survival of coliform in natural water bodies. In general, rates of biochemical reactions, and thus microbial growth rates, tend to increase as temperature rise. High growth rates place added demands on nutrient reserves which may not be renewed in dilute, natural systems, leading to an increase in death rates.

2.3.2 pH

Both acidity and alkalinity increase the bacterial death rate in laboratory tests, but under stream conditions the specific contribution of pH is not definable except when there is pronounced deviation from neutrality.

2.3.3 Effect of Nutrient

The dividing line between starvation and nutrients for multiplication is ill defined in the stream environment. Seldom is stream pollution of such character and magnitude as to stimulate sufficient growth for a net change in death rate to be detectable in the stream environment.

2.3.4 Sedimentation and Adsorption

Adsorption, coagulation, and flocculation may affect coliform disappearance rate, although few quantitative data are available. Adsorption refers to the attachment of coliform organisms to suspended particles. Coagulation refers to the coalescence of bacteria into clumps, and flocculation refers to the formation of soft loose aggregates incorporating much water.

According to Mitchell and Chamberlin (1978) early investigation by several workers have demonstrated that clays tend to absorb coliform more than do silts and sands. This is, of course, commonly the case with sorbed substances. As Mitchell and Chamberlin pointed out, the nature and stability of coliform aggregates incorporating other particulate matter depends to a very large extent upon the physiochemical nature of the particles. Ganon et al. (1983) found that 90 to 96 percent of the coliforms entering a lake from upland watersheds were associated with 0.45 to 5 μm particles.

Sedimentation may be a major mechanism responsible for the disappearance of faecal coliform bacteria from surface waters (Mitchell and Chamberlin, 1978; Gannon et al., 1983). Cells settle from the water column as discrete entities and as part of larger aggregates of faecal material, storm water debris and other suspended solids (Schillinger and Gannon, 1982). Gannon et al. (1983) observed that viable faecal coliform bacteria accumulated at the sediment surface in Ford lake, Mich., and concluded that sedimentation played an important role in the overall disappearance of faecal coliform bacteria from the water column.

However, turbid conditions associated with high stream runoff usually show a net increase in bacterial concentration by virtue of contamination flushed by surface wash from the drainage area. Subsequent sedimentation with adsorption or entrapment on the receding hydrograph may induce a decrease in bacterial concentration to a point below that expected for the time of passage in the death rate.

2.3.5 Contact Opportunity and Biological Extraction

Small shallow streams afford greater biological contact opportunity for bacterial extraction than large, deep rivers, and they usually show higher death rates.

2.3.6 Effect of Competitive Life

Perhaps one of the most potent factors beyond the element of time of passage in the death rate of coliform bacteria in the stream environment is the presence of competitive life. The natural biological life of rivers is much too rugged for the survival of organisms whose normal habitat is the shelter of the intestinal tract of man and other warm blooded animals. That competition from other biological life plays a significant role is evidenced in the greatly retarded death rate of coliform bacteria placed in sterile water in comparison with the death rate of coliform bacteria placed in natural fresh water or sea water.

2.3.7 Effect of Sea Water Salinity

Most of the investigations of effect of sea water on the death rate of coliform are based on laboratory results as it is difficult to measure reliably the time of exposure in the open ocean. However in the river environment interest is in the brackish waters formed by large fresh water rivers discharging into an ocean. Since 100% sea water has a specific gravity of 1.03, a mixture of sea water and fresh water extends upstream a considerable distance to a point of 100% freshwater; the stretch in between comprises the brackish reach. A further complicating factor is the web and flood translation induced by tides.

2.3.8 Effect of Sunlight

The degree of penetration of sunlight into the water column have a significant effect on many areas of water quality including bactericidal effect. Bactericidal effects are strongest in the ultraviolet region (near 260 nm; Lantrip, 1983), however, visible irradiance has also been shown to be an agent in coliform mortality (McCambridge and McMeekin, 1981). In fact, visible wavelengths may take on particular significance in natural streams because (i) u.v. wavelengths represent a small fraction (<3%) of total incident radiation (Lantrip, 1983) and (ii) u.v. radiation is rapidly attenuated in the water column, especially when dissolved organic matter is present .

The effect of sunlight on the decay of faecal coliform has been evaluated in a variety of studies including the work of Gameson and Gould (1974). According to them

$$k_{BO}(t) = \alpha I_O(t) \tag{2.1}$$

where $K_{BO}(day^{-1})$ is the decay rate at the surface, α is a proportionality constant, and $I_O(t)$ is the solar radiation in cal/cm^2.hr.

However, it must be recognised that solar radiation varies with depth as function of the light extinction coefficient. The two principal mechanisms for the extinction of solar radiation are absorption and scattering. In the former, short wave energy is transferred to long wave energy. The presence of particles in the water may also absorb the light. Scattering in water is the effect of reflection and diffraction by particles and in pure water due to small density fluctuations and other factors.

The degree of solar radiation penetration, therefore, depends on several factors: non-volatile suspended solids, organic detritus, and living particulates such as phytoplankton. The intensity of solar radiation and its angle with respect to the water surface are also important. Further, different regions of the incoming solar radiation spectrum (i.e., from infrared to ultraviolet) may be selectively absorbed or scattered.

The penetration (or conversely its extinction) of incoming solar radiation can be described by introducing the extinction coefficient. It has been observed that the extinction of light is proportional to the light at any depth. Therefore, a differential equation that expresses this observation is

$$\frac{dI}{dz} = -K_e I \tag{2.2}$$

where I is the solar radiation in $cal/cm^2.min$, z is the depth in m, K_e is the overall extinction coefficient in $meter^{-1}$. The above equation when solved for the boundary condition $I = I_0$ at $z=0$, becomes

$$I = I_0 \exp(-K_e z) \tag{2.3}$$

By integrating equation 2.3 and using the result in equation 2.1, the depth averaged effect of sunlight on decay rate can be shown to be

$$\overline{K_B} = \frac{\alpha I_0(t)}{HK_e}[1 - \exp(-K_e H)] \tag{2.4}$$

where H is the depth in m over which the average is taken and K_e (m^{-1}) is the vertical light extinction coefficient.

2.3.9 Effect of Bottom Sediments as Reservoir of Organisms

It is known for quite some time that organisms in the microscopic range can adhere to particles dispersed in water and waste water. Thus, the discharge of bacteria and viruses to natural waters may result in the sorption of such organisms to particles. As the particles settle into bottom sediments, microorganisms also accumulate. Apparently microorganisms can survive in the sediments for longer periods of time than in the overlying water column. Hence samples of bottom sediment yield more bacteriological evidence of the degree of faecal pollution than either water or oysters.

Since the sediment may include large concentrations of microorganisms, the Resuspension of such sediment and subsequent desorption may be an important source of contamination in the overlying waters.

2.4 KINETICS OF SURVIVAL OF FAECAL COLIFORM

The fate of bacteria in an unfavourable environment was first studied by Chick (Velz, 1984). She stated that bacteria die at a constant rate, i.e., a given percentage of residual population dies during each successive time unit. The mathematical formulation is as follows:

$$\frac{dN}{dt} = KN$$

$$\Rightarrow \log_e \frac{N}{N_o} = -Kt \tag{2.5}$$

$$\Rightarrow \log_{10} \frac{N}{N_o} = -K_1 t$$

where N_0 is the initial number, N is the residual after any time t, and K_1 is the reaction, or death rate.

A substantial body of laboratory and field investigation strongly suggests that the survival of pathogens and nonpathogens of special interest in stream sanitation approximate Chick's law.

2.4.1 Death Rate and Survival of Faecal Coliform in the Stream Environment

If the overall net first-order decay rate of faecal coliform is designated as K_r (per day), then the principal components of the net decay rate can be written as

$$K_r = K_d + K_i + K_s - K_g \qquad\qquad (2.6)$$

where, K_d = death rate as a function of temperature, salinity, predation etc.

K_i = death rate due to irradiance

K_s = net loss due to settling and resuspension (may be positive or negative)

K_g = after growth rate

Two representations of the loss rate can be utilised. The first and the most simple model uses the over all net loss rate K_r as the measure of bacterial kinetics and no attempt is made to describe the individual mechanisms or the kinetic structure (Velz, 1984). This simple model recognises that there may be considerable uncertainty in the input loads in certain problem contexts and that it is really not practical or meaningful to describe the kinetic structure at any significant level of detail.

The second level of models incorporates some of the principal mechanisms discussed above. Most of the models use the effect of salinity, temperature and solar radiation (Mancini, 1978; Thoman, et al., 1987). The general expression of these models are as follows:

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$$K_r = [K_{20} + K_{sl}]\theta^{T-20} + \frac{\alpha I_o(t)}{K_e H}[1 - \exp(-K_c H)] + \frac{v_s}{H} \qquad (2.7)$$

Here v_s is the net loss rate in units of length/time of the particulate coliforms. v_s can be positive, zero, or negative depending on the degree of resuspension. K_{sl} is the decay rate due to salinity. The increased complexity of this formulation of K_r is worthwhile in the situations where input loads are known with some degree of certainty.

2.4.2 Loss Kinetics of Faecal Coliform in Lakes

The overall loss rate of faecal coliform in the lake environment (K_l) can be shown to be the collective effect of three mechanisms, i.e.,

$$K_l = K_d + K_i + K_s \qquad (2.8)$$

where, K_d = rate coefficient for death in the dark; includes effects of

temperature, salinity, predation,etc. (day^{-1}).

K_i = rate coefficient for death as mediated by irradiance. (day^{-1})

K_s = rate coefficient for sedimentation loss. (day^{-1})

CHAPTER 3
METHODOLOGY

3.1 INTRODUCTION

The objective of this study was to acquire a better understanding of the decay mechanism of faecal coliform in aquatic environment and to assess the microbial self-purification capacity of some surface water bodies. In this chapter the procedures followed to achieve these goals are described. A description of the methods used is also included.

3.2 EXPERIMENTAL APPROACH

3.2.1 Decay Kinetics

Enteric pathogens and faecal coliform are not natural habitat of water bodies. They enter water sources directly through human or animal contact or indirectly via sewage input or urban or rural runoff. Thereafter various environmental factors act to inhibit their growth and survival. Typically a first order logarithmic expression (equation 2.5) is used to describe the decay kinetics of faecal coliform in aquatic environment. In this study an effort was made to examine the validity of this expression. For this purpose, the decay of · faecal coliform was observed in various types of water under controlled environmental conditions in the laboratory.

River water was collected from the river Buriganga. Lake water was collected from Dhanmondi lake. Sampling locations were chosen at a considerable distance from effluent discharge points so that the waters represent the overall

quality of respective water bodies. Samples were collected from the centre of the water bodies in sterilised 300-ml BOD bottle. The bottles were lowered about 6 cm below the water surface and then the caps were removed to allow entry of water into them. Samples were carried to the laboratories in a wooden box to eliminate the effect of direct sunlight.

For comparison decay of faecal coliform was also observed in sterilised distilled water. Distilled water was sterilised in autoclave at 121°C temperature and 15 psi pressure for 20 minutes. Samples were prepared by mixing fresh sewage with sterilised distilled water.

Samples were preserved in 300-ml bottles under room temperature. The room temperature varied from 26°C to 28°C. Faecal coliform concentration was measured daily until the decay pattern became apparent. The membrane filter technique was used for enumeration of faecal coliform. To minimise statistical error three dilutions of each sample were made and the mean value was taken.

3.2.2 Effect of Environmental Factors

Various factors are known to influence the decay of faecal coliform in aquatic environment. Primary among such factors are temperature, irradiance, predation, nutrient deficiency, pH, sedimentation and biological extraction. In this study an effort was made to determine the effect of these parameters on the survival of faecal coliform. However, due time and resource constraint only the following parameters were considered:

    i.   Temperature

    ii. pH

iii. Nutrients

The above parameters often vary in natural waters and the study is likely to lead to a better understanding of the decay mechanism of faecal coliform in natural water environment.

To determine the effect of temperature on the decay of faecal coliform the decay rate was observed at five different temperatures in the range varying from below $4^{\circ}C$ to $49^{\circ}C$. For each temperature two 300 ml samples were prepared by mixing fresh sewage with sterilised distilled water. Incubators were used to maintain a constant temperature. The decay rate below $4^{0}C$ was observed by placing two samples in the refrigerator. Faecal coliform concentration of each sample was measured daily until a stable decay rate was obtained. The membrane filter technique was used for enumeration of faecal coliform. To minimise statistical error 3 dilutions of each sample were made and the mean value was taken.

To determine the effect of pH on the decay of faecal coliform, the decay rate was observed at five pH level between 4.0 and 9.0. The samples were prepared by mixing fresh sewage with 300 ml of sterilised distilled water. To maintain constant pH, buffers were used, which resist radical changes in the hydrogen ion concentration when acid or alkali is produced in the medium. Death rates were calculated over periods when the population was clearly in decline to avoid interference from after-growth effects in the early stage of incubation.

The nutrients that are important for the growth and survival of micro-organisms in aquatic environment are carbon, nitrogen and phosphorous. In most cases however, nitrogen and phosphorous are the nutrients that limit

growth. Nitrogen is available in three forms-- ammonia (NH_4), nitrate (NO_3) and nitrite (NO_2). Phosphorous is mainly available as phosphate (PO_4). To investigate the effect of nutrients on the survival of faecal coliform, its decay was observed at three different levels of nutrients. The samples were prepared by adding nutrient media to sterilised distilled water and then fresh sewage was inoculated into it. For each nutrient level two samples were prepared and were kept in room temperature in 300-ml BOD bottle. Nutrient concentration was measured at the beginning of the test. Faecal coliform concentration was measured using the same membrane filter method until the population was clearly in decline.

In addition an attempt was made to assess the microbial self-purification capacity of natural water bodies and hence the loss rate of faecal coliform was measured in actual stream and lake environment.

In the following sections the methods of enumeration of faecal coliform in the laboratory and the methods of measuring the loss rate of faecal coliform in streams and lakes is described.

3.3 ENUMERATION OF FAECAL COLIFORM

There are two standard methods for detection and enumeration of indicator organism (APHA, 1985).

    i.   Multiple Tube Method

    ii.   Membrane Filter Method

These two methods are described in the following paragraphs.

3.3.1 Multiple Tube Method

In the multiple tube (MT) method different amounts of water are added to tubes containing suitable culture media. The bacteria present in the water reproduce, and in accordance with the number inoculated and the number of tubes with a positive reaction, the most probable number (MPN) of bacteria present in the original water sample can then be statistically determined.

The multiple tube method is applicable to all kinds of water samples: it can cope with clean, coloured, or turbid waters containing sewage or sewage sludge, or mud and soil particles, provided that the bacteria are homogeneously distributed in the prepared test samples. Theoretically the technique is sufficiently sensitive to measure low levels of bacteria in water samples, although bigger containers to take larger volumes of sample are then needed to be used as culture vessels; however for normal water analysis, 10 ml is usually the largest volume used. This method is often preferred by microbiologists.

3.3.1.1 Culture Media

Commercially available dehydrated media simplify the preparation of culture broth, and are therefore recommended for laboratory work. Various manufacturers produce these media as powders, which can then be easily weighed out, dissolved in distilled water, and dispensed into culture tubes prior to sterilisation.

Several different culture media are available for the presumptive test, for example:

- lauryl tryptose broth (LTB);
- MacConkey broth;
- lactose broth

These three media are in common use in many countries. The selectivity of MacConkey broth and LTB depends respectively on the presence of bile salts and the surface-active agent, lauryl sulphate. Lactose broth is a non-selective medium.

As a confirmatory medium for total coliforms brilliant green lactose bile broth (BGB) is most widely used.

To confirm the presence of faecal coliforms, either BGB broth or Escherichia coli (EC) broth is used.

Preparation of media: Media should be prepared in accordance with the manufacturer's instructions. The general practice is described below.

i.   A given amount of dehydrated medium is dissolved in distilled water to obtain the double-strength or single-strength presumptive medium (for confirmatory analysis, only single strength medium is used).

ii.   Requisite volume of medium is dispensed into culture tube containing an inverted Durham tube and the tube is then capped.

iii.   Culture tubes are then sterilised in an autoclave or pressure cooker at 114°C for 10 minutes (or in accordance with the manufacturer's specifications). It is particularly important that media containing disaccharide, e.g. lactose, are not autoclaved at higher temperatures.

iv.   The sterilised medium should be stored at room temperature (approximately), to maintain sterility. In addition, since several dyes are light-sensitive, the solution should be protected from exposure to light.

3.3.1.2 Test Procedure

Figure 3.1 shows the procedures involved in Multiple Tube test of water and wastewater sample, together with the appropriate incubation times and temperatures. The procedure to be used for testing relatively unpolluted water, such as treated water form waterworks, is described below.

i.   The paper wrapping is removed from the sample bottle.

ii.   With the stopper in position, the bottle is shaken vigorously to achieve a homogeneous dispersion of bacteria. (If the bottle is completely full of water, about 20-30 ml of water is discarded from the bottle prior to shaking. This ensures thorough mixing).

iii.   With a sterile 10 ml pipette, 10 ml of the sample is inoculated into each of five tubes containing 10 ml of presumptive broth (double strength). It is advisable to shake the tubes gently to distribute the sample uniformly throughout the medium.

iv.   The tubes are then incubated at 35°C or 37°C for 24 hours.

v.   At the end of the 24-hour incubation period, each tube is examined for the presence of gas. Gas, if present, can be seen in the Durham tube; if none

```
                         ┌─────────────┐
                         │ Water sample│
                         └─────────────┘
                      24 h at 35°C or 37°C

        ┌────────────────────────┴────────────────────────┐
    ┌────────┐                                         ┌──────┐
    │ No gas │                                         │ Gas  │
    └────────┘                                         └──────┘
    Reincubate for
       24 h at
    35°C or 37°C

   ┌──────────┴──────────┐        ┌──────────────────────────┐
┌────────┐           ┌──────┐     │ Positive Presumptive test│
│ No gas │           │ Gas  │     │      for coliforms       │
└────────┘           └──────┘     └──────────────────────────┘
     │
┌─────────────┐
│NEGATIVE FOR │
│ COLIFORMS   │
└─────────────┘
```

┌──────────────┐
│Confirmed test│
└──────────────┘

```
  ┌─────────────────────────┐        ┌──────────────────────┐
  │ 48 h at  35°C or 37°C    │        │  24 h at 44°C        │
  └─────────────────────────┘        └──────────────────────┘

  ┌──────────┴──────────┐             ┌──────────┴──────────┐
┌────────┐         ┌──────┐        ┌────────┐           ┌──────┐
│ No gas │         │ Gas  │        │ No Gas │           │ Gas  │
└────────┘         └──────┘        └────────┘           └──────┘
     │                 │                │                    │
┌─────────────┐  ┌─────────────┐  ┌────────────────┐  ┌────────────────┐
│NEGATIVE FOR │  │POSITIVE FOR │  │NEGATIVE FOR    │  │POSITIVE  FOR   │
│COLIFORMS    │  │COLIFOFORMS  │  │FAECAL COLIFORMS│  │FAECAL COLIFORMS│
└─────────────┘  └─────────────┘  └────────────────┘  └────────────────┘
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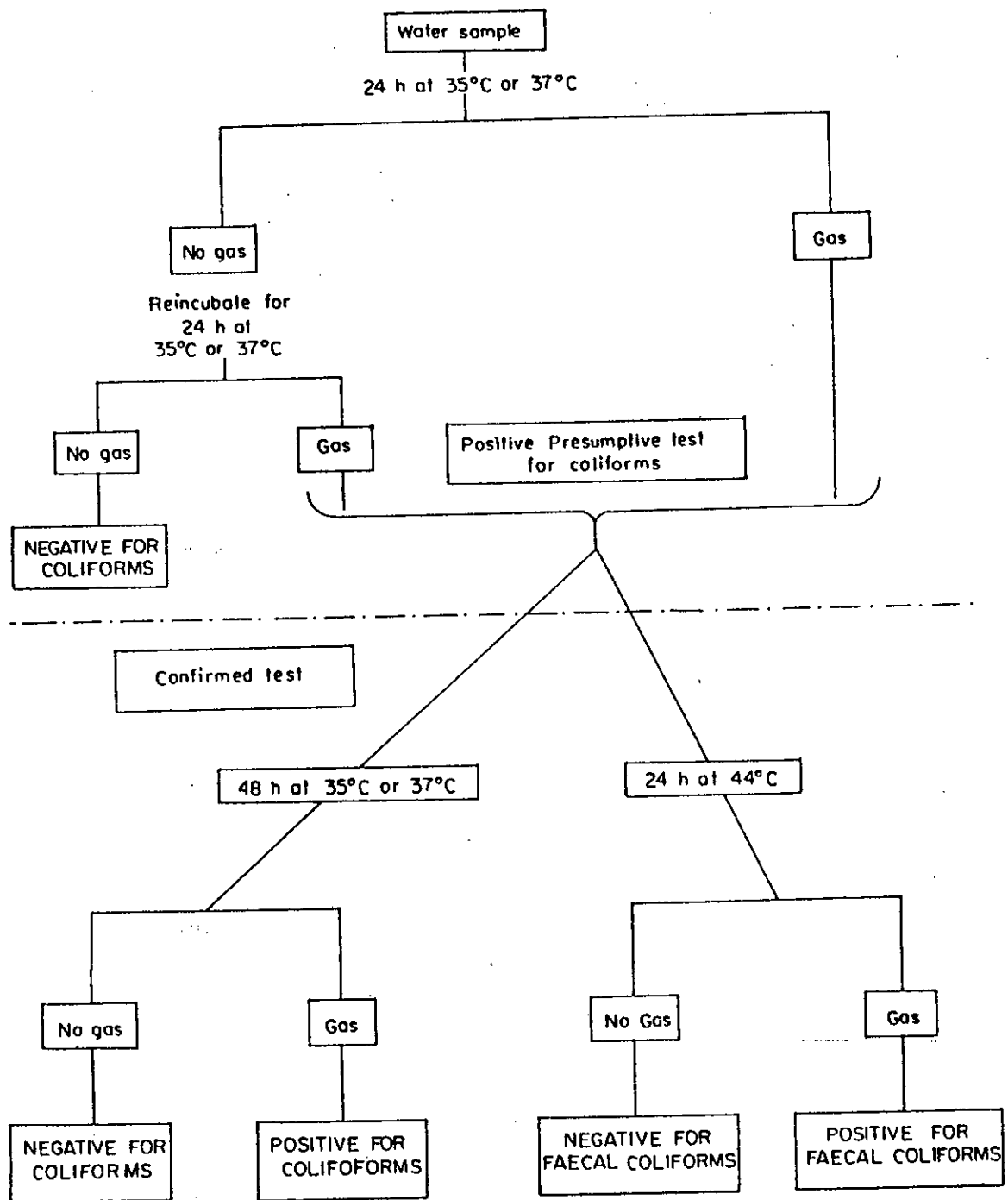
Fig. 3.1 Procedures involved in presumptive and confirmed multiple tube tests

is visible, the tube is gently shaken. If any effervescence (streams of tiny bubbles) is observed, the tube should be considered positive.

vi. The number of positive tubes after 24 hours is recorded in a table.

vii. Negative tubes are kept in incubator for a further 24-hour period. At the end of this period, the tubes are again examined for gas production as described before. Gas production at the end of either 24 or 48 hours incubation is presumed to be due to the presence of coliforms in the sample.

viii. The number of positive tubes after 48 hours is recorded in the table.

ix. The confirmed test should be carried out at the end of both the 24-hour and the 48-hour incubation. Using a loop, one or two drops from each presumptive positive tube is transferred to a corresponding sterile confirmative 10-ml tube containing, e.g. BGB broth. Before each transfer, the inoculation loop is sterilised by flaming and is then allowed to cool.

x. If the presence of faecal coliforms is also to be investigated, subcultures in two tubes containing confirmative broth (e.g. BGB broth) should be prepared from each presumptive positive tube. EC medium is preferred in some areas for confirmation of faecal coliforms.

xi. To confirm the presence of coliforms, one subculture tube is incubated from each presumptive positive tube for 48 hours at 35°C or 37°C.

xii. The tubes are examined at the end of the 48 hour incubation period; the presence of gas confirms that coliforms are present in the sample. The results recorded in the table.

xiii. To confirm the presence of faecal coliforms a second subculture tube is incubated form each presumptive positive tube for 24 hours at 44 ± 0.5°C.

xiv. If at the end of 24 hours incubation, gas is found in the tubes, the presence of faecal coliforms is confined.

3.3.1.3 Determination of MPN

The multiple-tube method allows one to estimate the density of presumed coliforms in the sample with an accuracy that increases, as do all statistical estimates, with the number of replicates examined. Results are expressed as MPN units per 100 ml, where MPN means the most probable number and reflects the statistical basis of the estimate. Only a series of replicates in which some tubes have positive and some tubes have negative results should be used to estimate numbers, since if all tubes are positive, one cannot know whether the initial number of cells placed in each tube was small or very large. The statistical estimate of the average number of coliforms per tube is based on the Poisson distribution

$$P(X) = \frac{m^X e^{-m}}{X!}$$

where P(X) is the probability of X, m is the mean, and X! is defined as 1 when X=0. In a dilution end-point assay, such as multiple-tube assay for coliforms, the average number of cells per tube can be calculated from the probability (frequency) of 0 cells per tube, i.e., from the frequency of negative tubes in a series of replicates giving both positive and negative results. In this case,

$$P(0) = e^{-m}$$

and m, the average number of coliforms per volume of sample used, is the natural logarithm of the fraction of negative tubes, P(0). Tables are available for the determination of MPN. For treated water, where five 10 ml portions are inoculated, the MPN can be found using Table 3.1.

Table 3.1 MPN of various combinations of positive and negative results when five 10-ml portions are used.

Number of tubes giving positive reaction out of 5 tubes of 10-Ml each	MPN
0	0
1	2.2
2	5.1
3	9.2
4	16.0
5	indeterminate

An example is given in Figure 3.2 to show how the test results are interpreted. It will be seen that three confirmed positive tubes have been obtained for the total coliform group. From Table 3.1, the corresponding value of the MPN can be seen to be 9.2 total coliforms per 100 ml sample. As regards the test for faecal coliforms, there was only one confirmed positive tube. Consequently, the value of the MPN for this group is 2.2 per 100 ml.

A PRESUMPTIVE TEST

Inoculate 10 mL of sample into each of 5 tubes containing presumptive broth. Incubate for 24 h at 35°C or 37°C

GAS positive presumptive test

NO GAS incubate for another 24h (total incubation time 48 h)

GAS positive presumptive test

NO GAS coliforms absent

B. CONFIRMED TEST FOR TOTAL COLIFORMS AND FAECAL COLIFORMS

Inoculate two tubes of broth (e.g., BGB broth) from each positive presumtive tube

Incubate for 48 h at 35°C or 37°C

Incubate for 24 h at 44°C

GAS total coliform positive

NO GAS total coliform negative

GAS faecal coliform positive

NO GAS faecal coliform negative

CONFIRMED POSITIVE TEST

CONFIRMED NEGATIVE TEST

CONFIRMED POSITIVE TEST

CONFIRMED NEGATIVE TEST

Fig. 3.2 Example of determination total coliforms and faecal coliforms

3.3.2 Membrane Filter Method

In contrast to the multiple-tube (MT) method, the membrane filter (MF) method gives a direct count of total coliforms and faecal coliforms present in a given sample of water. The method is based on the filtration of known volume of water through a membrane filter consisting of a cellulose compound with a uniform pore diameter of 0.45 μm; the bacteria are retained on the surface of the membrane filter. When the membrane containing the bacteria is incubated in a sterile container at an appropriate temperature with a selective differential culture medium, characteristic colonies of coliforms and faecal coliforms develop, which can be counted directly. This technique is popular with environmental engineers.

The specific advantages of this method are:

- results are obtained more quickly as the number of coliforms can be assessed in less than 24 hours.
- gives direct results;
- requires less supplies and glassware;
- easy to use in laboratories, or even in the field if portable equipment is used.

However this method also have the following drawbacks:

- high turbidity caused by clay, algae, etc. prevents the filtration of a sufficient volume for analysis and it may also produce a deposit on the membrane which could interfere with bacterial growth;

- presence of a relatively high non-coliform count may interfere with the determination of coliforms;

- water containing particular toxic substances which may be absorbed by the membranes, can affect the growth of the colliforms.

3.3.2.1 Field Equipment for MF Method

(I) Incubator; (ii) Rack for incubation of petridishes; (iii) Filter funnel, 100 ml capacity; (iv) Porous support for filter; (v) Filter support; (vi) Suction vessel; (vii) Syringe with two way valve for providing the vacuum needed for the filtration; (viii) Sample vessel; (ix) Forceps, (x) Bottle containing alcohol; (xi) Bottle containing sterile buffer; (xii) Plastic petridishes; (xiii) Pipettes.

3.3.2.2 Volume of Water Sample for Filtration

Since the filtration area is relatively small, it can only support the growth of a limited number of colonies. The optimum number is between 20 and 80 colonies, with a maximum of 200. If this figure is exceeded, very small a typical colonies or superimposed colonies may develop, or growth inhibition due to overpopulation may result.

The volume of sample to be filtered depends on the extent of work and type of water. If the origin of the sample is unknown and its probable bacterial content is uncertain, water volumes differing by a factor of ten should be filtered in order to find the appropriate range for the analysis. If the volume to be filtered is less than 10 ml, at least 20 ml of sterile dilution water should be placed in the funnel prior to the filtration.

The following test volumes are suggested by the Environmental Protection Agency of USA (Table 3.2).

Table 3.2 Recommended Sample Volumes for Indicator Organisms

Organism	Type of Water	Sample Volumes	Range/ml
Total Coliform	Unpolluted, raw, surface	1,4,15,60	33-8000
	Polluted, raw, surface	0.02,0.08,0.15,0.5	4000-400000
	Sewage, diluted sewage	0.0003,0.001,0.003, 0.01	200000-27000000
Faecal Coliform	Unpolluted, raw, surface	1,3,10,30	67-6000
	Polluted, raw, surface	0.1,0.3,1.0,3.0	670-60000
	Sewage, diluted sewage	0.0003,0.001,0.003	670000-20000000
Faecal Streptococci	Unpolluted, raw, surface	1,5,25,100	20-10000
	Polluted, raw, surface	0.1,0.5,2.0	1000-100000
	Sewage, diluted sewage	0.2,1.0,5.0	400-50000

3.3.2.3 Culture Media

Various media can be used for the examination of coliform organisms by the membrane filtration method. Of these lactose tergitol agar, lactose TTC,

tergitol agar and lauryl sulphate lactose broth may be used for coliform counts at 35°C or 37°C, and MFC broth at 44°C for faecal coliform counts. Although all these media rely on the fermentation of lactose for the detection of presumptive coliform organisms, the characteristic reaction varies with each medium. The characteristic metallic sheen of colonies on Endo-type media depends on the formation of aldehyde.

Although it is possible to prepare the media from the basic ingredients, this may be impractical for a small laboratory. The use of dehydrated media is therefore recommended. The media can be prepared as a broth, and used together with nutrient absorption pads, or as solid agar plates. The broth may be solidified by the addition of 1.2-1.5% again before boiling.

Preparing Media:

Sterile Phosphate Buffer water

i.   34.0 gm of potassium dihydrogen phosphate (KH_2PO_4) is dissolved in 500 ml of distilled water.

ii.   It is then diluted to 1,000 ml with distilled water to produce 1 litre of stock buffer solution. Stock buffer is stored in refrigerator and is discarded if it becomes turbid.

iii.   1.25 ml of stock buffer solution and 5 ml magnesium sulphate is added to a litre of distilled water to make 1 litre of phosphate buffer water.

iv.   The solution is sterilised by filtering through a Millipore GS (0.22 μm pore size) membrane filter, or by autoclaving in loosely capped container at 121°C for 15 minutes at 15 psi.

v.   The solution is stored in tightly capped containers on a cool, dark shelf or in a refrigerator.

Preparation of stock MF-C Medium

i.   100 ml of distilled water is added to 3.7 gm of dehydrated MF-C medium in a 250 ml screw cap Erlenmeyer flask.

ii.   In a separate flask, 100 ml of 0.2N NaOH solution is added to 1 gm of bacto-rosolic acid dehydrate to produce a 1% rosolic acid solution. For smaller quantities, 0.1 gm rosolic acid is added to 10 ml of 0.2N NaOH.

iii.   1 ml of 1% rosolic acid solution added into the rehydrated MF-C broth.

iv.   The medium is heated to the boiling point in a loosely covered flask; and then promptly cooled to below 45°C.

v.   Final pH is adjusted around 7.4.

vi.   The media is dispensed at room temperature. The unused portion is stored at 2-10°C and is discarded after two weeks.

Preparing Dilution Blanks

Preparing dilution blanks is simple. The best procedure is to sterilise the buffer and its container all at once in an autoclave. For the hundred factor dilution, clean dilution bottles are used, and for the ten-factor dilution, clean screw-cap 15x150 mm culture tubes are used. For the bottles, 102 ml and for the tubes 9.5 ml of sterile buffer is poured in. The excess buffer evaporates in the autoclave.

The dilution blanks are placed in the autoclave for 15 minutes at 121°C. After they have reached room temperature, it is ensured that dilution blanks have 99 ± 2 ml or 9± 0.2 ml; then the sterile blanks is stored in a cool, dark place.

Preparing Sterile rinse water

If sufficient buffer rinse is not available, sterile rinse water can be prepared in the field immediately in the following manner. 50 ml of the water to be sampled (provided it is known to be free of chemical inhibitory agents) is sucked into a 50 ml plastic syringe; a Swinnex-47 mm plastic filter holder fitted with a sterile filter is attached with the syringe and the water is forced though the sterilising filter onto the inner walls of the filter-holder funnel.

3.3.2.4 Test Procedure

Different types of filtration units and equipment exist, and the test procedure varies with the types of units. The general procedure is described below.

Determination of Faecal Coliforms (FC)

i.   The Erlenmeyer (side-arm) flask is connected to the vacuum source (turned off) and the porous support is placed in position. If an electric pump is used, it is better to put a second flask between the Erlenmeyer and the vacuum source, this second flask acts as a water trap and thus protects the electric pump.

ii.   A Petridish is opened and a pad is placed on it.

iii.   With a sterile pipette 2 ml of selective broth medium (MF-C broth) is added to saturate the pad.

iv.   The filtration unit is assembled by placing a sterile membrane filter on the porous support, using forceps sterilised by flaming.

v.   the upper container is placed in position and is secured with the special clamps. (The type of clamping used depends on the type of equipment).

vi.   Optimal volume of sample is poured into the upper container. If the test sample is less than 10 ml, at least 20 ml of sterile dilution water should be added to the top container before filtration applying the vacuum.

vii.   After the sample has passed through the filter, the vacuum filter is disconnected and the container is rinsed with 20-30 ml of sterile dilution water. The rinsing operation is repeated after all the water from the first rinse has passed through the filter.

viii.   The filtration unit is dissembled and, using the forces, the membrane filter is placed in the Petridish on the pad with the grid side up. Care should be taken so that no air bubbles are trapped between the pad and the filter.

ix.   Petridish is inverted for incubation.

x.   The Petridish is incubated at 44±0.5°C for 18-24 hours with 100% humidity. Alternatively, tight-fitting or sealed Petridishes may be placed in water-proof plastic bags for incubation. The bags are then submerged in a water-bath maintained at 44±0.5°C or 24 hours. The plastic bags must be kept below the surface of the water throughout the incubation period. They can be held down by means of a suitable weight, e.g., a metal rack.

Colonies of faecal coliform bacteria are of blue colour. This colour may cover the entire colony, or appear only in the centre of the colony. Colonies of other colours should not be counted. The colonies can be counted with the aid of a lens. the number of faecal coliforms per 100 ml is then given by:

$$\text{Faecal coliform per100ml (cfu / 100ml)} = \frac{\text{no. of faecal coliform colonies counted}}{\text{no. of ml of sample filtered}}$$

3.3.2.5 Sterilisation Procedures

Proper sterilisation of equipment is of utmost importance when performing microbiological analysis. These procedures are necessary to insure that interfering organisms are kept to a minimum. the following section outlines

the sterilisation procedures that must be performed in order to obtain valid results.

Sampling Bottles and Pipettes

Sampling bottles made of borosilicate glass or heat resistant polypropylene having a minimum capacity of 120 ml should be used. If the water to be sampled is chlorinated then 0.1 ml of a 10% sodium thiosulfate solution, a dechlorinating agent, should be added to the bottle prior to sterilisation. The bottle is wrapped in Kraft paper and then autoclaved at 121°C and 15 psi for 15 minutes. If the bottle is heat resistant, dry heat at 170°C for 1 hour can be applied to sterilise it. Glass pipettes are sterilised by autoclaving at 121°C and 15 psi for 30 minutes, or by applying dry heat at 170°C for 1 hour.

Filter Holders

The filter holders can be sterilised by any of the following methods.

i.   Ultraviolet Light: This method is recommended for all Millipore filter holders because of its speed, safety, and efficient kill (99.9%). The EPA recommends a two minute exposure period for clean filter holders (AWWA,1990).

ii.   Steam Autoclaving: The funnel and base is wrapped in Kraft paper and autoclaved at 121°C and 15 psi for 15-20 minutes. Units remain sterile until opened.

iii.   Dry Heat: This is only recommended for the Pyrex holder. The stopper is removed and then wrapped in aluminium foil. Heat is then applied for 1 hour at 170°C. Units remain sterile until opened.

The filter holders can be sanitised by any one of the following methods:

i.   Boiling: Any holder can be sanitised just prior to use by immersion in boiling water for 3.5 minutes.

ii.   70% Alcohol immersion and drying: This procedure is recommended only when other methods are not available. The filter holder is immersed for a few minutes, and then is allowed to air dry. This works with any holder.

Phosphate buffer

i.   Autoclave technique: For rinse buffer, the solution is either kept in a litre bottle or is dispensed into 100 ml bottles. For dilution flasks, the amount of sample should be such that it would provide 99 ± 2.0 ml or 9.0 ± 0.2 ml after autoclaving. The solution should be loosely capped or covered and then autoclaved at 121°C and 15 psi for at least 20 minutes.

ii. Membrane filter technique: If an autoclave is not available, but an oven is, a one litre empty flask is sterilised for 1 hour at 170°C. A sterile filter holder and a sterile HA filter is installed, and buffer is processed via vacuum filtration. It may be necessary to use a prefilter (AP20) to prevent membrane clogging. The buffer may be either dispensed from the side arm or transferred to another sterile bottle. The buffer should not be stored for long period of time.

If an autoclave and oven are not available, the buffer is processed as above to remove particles. Then the buffer is dispensed directly into the filter funnel or dilution flask as needed with a plastic syringe and attached sterile Millex filter unit.

Membrane filtration of buffer is actually better than autoclaving because dead bacteria and all particles, as well as live bacteria, are removed.

3.3.3 Reasons For Selecting Membrane Filter Method

Since its inception, membrane filter method has gained world-wide acceptance because of its high degree of reproducibility, its ability of testing relatively larger volume of sample and for the savings in time to gain definite result. The following advantages are cited by the National Training Centre of the U.S. Environmental Protection Agency (EPA).

- Results are obtained in approximately 24 hours, as compared with 48-96 hours for the standard fermentation tube method.

- Much larger, and hence more representative, samples of water can be sampled routinely with membrane filters.

- Numerical results from membrane filters have much greater precision (reproducibility) than is expected with the fermentation tube method.

- The equipment and supplies required are not bulky. A great many samples can be examined with minimum requirements for laboratory space, equipment, and supplies.

- The MF technique costs about half as much as the MPN method per test. This means considerable saving to any laboratory routinely performing coliform analyses.

- It is the only approved coliform test that lends itself to field testing.

- The alternative MPN method requires laboratory facilities for washing, autoclaving and incubation of many fermentation tubes, besides requiring many litres of culture media.

For the above mentioned advantages, the multiple tube method was selected for faecal coliform analyses.

3.4 METHODS OF MEASUREMENT OF DECAY RATE OF FAECAL COLIFORM IN THE STREAM ENVIRONMENT

Estimates of the coliform disappearance rate (K_r) may be obtained in a number of ways in the laboratory chamber studies, or, preferably in situ. For laboratory estimates, samples of effluent may be taken along with samples of receiving water. Then, under controlled conditions of light, temperature, and dilution, the time rate of disappearance maybe determined for various combinations of conditions. Unfortunately "bottle effects" often distort laboratory results.

In situ K_r values can be determined whether the flow regime is well defined or not, although there are inherent errors involved in each methods. Where there are no flow regime data, or where flows are of a transient nature, a commonly used method (Ganon et al., 1983) is to add a slug of conservative tracer substance (a dye, rare element, or radio isotope) to the steady-state discharge. Then the discharge plume is sampled, dilution is estimated from

concentrations of tracer and the dilution corrected coliform counts permit k to be estimated. It should be recognised that this technique may give misleading results where the dilution of the tracer is due to mixing with water heavily contaminated with the same discharge. Since the tracer had been introduced as a slug, there is no way to know how much of the surviving coliforms originated in the tracer-dosed effluent and how much came from pre-dosing or post-dosing effluent. However, where the flow regime is sufficiently predictable and stable to assure that dilution occurs essentially with ambient water, and where coliform levels in the ambient water are known, this should not be a problem.

Another method, which is particularly useful where discharge is to a channel, is as follows. First a base sampling site is established below the discharge where the water column is fully mixed normal to the direction of flow. Then samples are taken at the base site and at several points downstream. based upon known velocities and the change in coliform concentration with distance (time of travel), K_r values may be estimated. Clearly errors will be introduced to the extent that there is incomplete lateral mixing of the stream, nonuniform longitudinal velocities laterally and vertically across the channel, and unknown inflows causing dilution or introducing additional coliforms between sampling sites.

Also, sampling can be done so that the same parcel of water is sampled in case the discharge is not at steady-state. For example, if the first sampling site is one mile below the base site, and the channel flow has a mean velocity of 2 ft per second, then the first sampling site should be sampled:

$$\frac{5280}{mile} * \frac{1\ second}{2\ ft} * \frac{1\ hr}{3600\ seconds} = 0.73\ hrs$$

or 44 minutes after sampling at the base site. Clearly, however, this does not account for dispersion, and the 44 minutes is an average figure corresponding to the peak loading. Where possible, dye studies or other techniques should be used to characterise stream dispersion at the sampling location. Then, by integrating under the curve total surviving coliforms can be estimated. If, on the other hand, discharge and stream conditions are clearly at steady-state, sampling times are of no consequences.

3.4.1 Time of Passage

Time of passage from reach to reach along the watercourse is an essential parameter along stream self purification computations involving degradable wastes such as microbial waste. For calculation of time of passage, generally the steady-state Lagrangian concept is adopted, wherein the time of passage from cross-section to cross-section along the watercourse is taken as occupied channel volume displaced in blocklike manner by the tributary streamflow: t=CV/Q, where t is time of passage in second, CV is occupied channel volume in ft^3, and Q is the tributary flow in ft^3/sec.

In considering the time of passage along the course of a stream, a distinction is made between the traverse of the hydraulic crest, the time at which the changes in the rate of discharge are noted at locations down river, and the mass movement of the body of water. It is the latter that is the measure of the time of passage of wastes from reach to reach along the course. The hydraulic crest, more in the nature of translation of a surface wave, out strips movement of waste contained in the body of the water, particularly where large

volumetric displacements are involved through deep reaches or pools behind dams.

3.4.2 Adjustment for Tidal River

The expression of the time of passage implies blocklike movement of the body of water, with practically complete vertical and transverse mixing throughout the cross-sections but no appreciable longitudinal mixing. This simplified concept is applicable to most tidal rivers with minor modification. Blocklike movement of the water mass is characteristic of tidal rivers, and extensive tidal longitudinal mixing does not occur; the steep water quality gradients observed in polluted tidal rivers confirm these similarities to inland streamflow. If longitudinal mixing dominated, steep gradients would be levelled and the observed water quality would show more uniform conditions over long reaches. Obviously with reversal of tidal currents some longitudinal mixing does occur in short reaches but not with the completeness assumed in some theoretical approaches. The more acceptable concept, therefore, is blocklike movement.

The net movement from reach to reach toward the sea is brought about by the displacement of occupied channel volume by the discharge of landwater into the tidal reach. Since the occupied channel volume at mean tide remains quite fixed, it is axiomatic that with the continuous addition of land water runoff from the tributary drainage area an equivalent amount must find its way into the ocean; otherwise the channel level would continue to rise. Though a seaward movement takes place in steplike action as net access of ebb over flood translation, conversion to mean tide is equivalent to elimination of these oscillations, and movement through the tidal river may be considered as a

continuous forward net time of passage similar to that in freshwater stream: $T=V/Q$, where V is the occupied channel volume to mean tide, Q is the rate of landwater runoff, and T is the net time of passage.
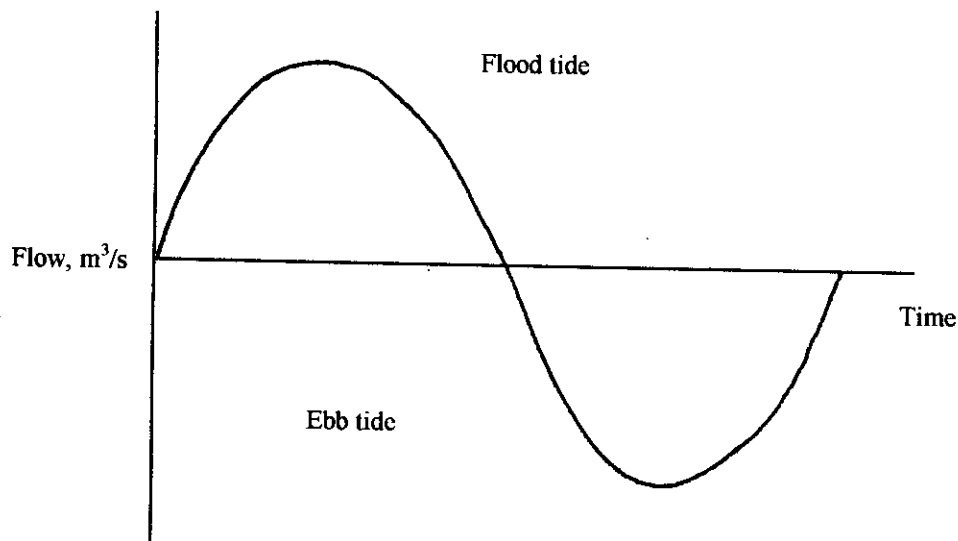
Fig. 3.3 Computation of net flow

3.4.3 Sampling

Meaningful and reliable sampling assures the validity of analytical findings. the shorter the time interval between collection of a sample and its analysis, the more reliable is the results. There must be no significant change in sample between the time of collection and analysis. Immediate field analysis is required for certain constituents and physical characteristics to assure dependable results, since changes in composition of the sample may occur in transit to the laboratory.

It is difficult to state exactly how much time can be allowed between the collection of a sample and its analysis. the following maximum limits are suggested in Standard Methods (APHA, 1985) as reasonable for physical and chemical analyses:

Unpolluted waters, 72 hrs;
slightly polluted waters, 48 hours; and
polluted waters, 12 hrs

The time and place of sampling and analysis should be recorded.

Configuration of the stream channel, irregularity of cross-section, depth, bends and gradient should be considered in locating a sampling station. Wherever possible a single sampling position in the cross-section is desirable, but the position must be representative of the main body of flow. Sampling from shore is seldom satisfactory, particularly in an irregular section with shallow beach and deep channel. A good location is a reach of straight, regular cross-section without excessive turbulence downstream of bends and irregular reaches that have produced good mixing and uniformity through the section. Bridges may afford a convenient location, if other features are satisfactory. Generally, however, a boat is necessary for most stations; A boat offers the advantage of flexibility - it is possible to check transversely at a given section and longitudinally between sections for the occurrence of any abnormalities.

3.5 METHODS OF MEASUREMENT OF DECAY RATE OF FAECAL COLIFORM IN LAKE ENVIRONMENT

Direct and in situ measurement of death rate of faecal coliform in actual lake environment is usually not possible. The general procedure is to separately measure the loss rate of faecal coliform due to various factors and then to add these loss rate to get the actual loss rate in the lake environment. A number of biotic and abiotic factors influence the faecal coliform death rate in lake environment, including algal toxins, bacteriophages, nutrients, pH, predation, temperature, salinity, irradiance and, temperature. However the general procedure is to measure the effect of only the major factors, i.e., irradiance, temperature and settling.

The collective effects of sedimentation and irradiance and temperature mediated death on the overall loss coefficient is expressed as

$$K_r = K_d + K_i + K_s \qquad\qquad (3.1)$$

where, K_d = rate coefficient for death in the dark; including effects of temperature, salinity, predation, etc. (day^{-1}).

K_i = rate coefficient for death as mediated by irradiance (day^{-1}).

K_s = rate coefficient for sedimentation loss (day^{-1}).

3.5.1 Measurement of Rate Coefficient for Death in the Dark

Death rate in the dark includes the effect of temperature, predation, salinity etc. It is better if this component can be measured in situ. However for various practical reasons, it is often not possible. Usually, the sample is collected

from the lake in a dark shrouded container. Care is taken so that the sample does not come into direct contact with sunlight. Before transportation to the laboratory the sample is usually kept in ice bag.

3.5.2 Measurement of Rate Coefficient for Sedimentation Loss

Settling losses in lakes are traditionally quantified by placing collection devices (traps) immediately below the thermocline. Transformations (e.g. death of bacteria) which may occur during incubation may distort results and make this technique inappropriate for the study of faecal coliform. A two-step approach is generally applied where

i. the association of faecal coliform bacteria with particles of various size classes is identified and

ii. the sedimentation rate of particles in each size class is quantified.

A serial screening technique (Schillinger and Ganon, 1982; Ganon et al., 1983) is used to identify the association of faecal coliform bacteria with particles of various size classes. Water samples are collected from the lake during storm overflow events. The samples are gently mixed, with care taken to avoid disintegration, and 100-ml aliquots is pored sequentially through a series of sterile mesh screens (102, 53, 20, 10, 6 and 1 μm) followed by a 0.45 μm membrane filter. Gravity flow is augmented with a brief vacuum application at 15 psi. The screens and filters are aseptically removed and placed into bottles containing 100-ml aliquots of sterile phosphate-buffered water with 5 drops of non-toxic non-ionic surfactant added to disperse bacteria from the particles and the screens. The bottles are shaken for 5 minutes to remove bacteria from the screens, insure disintegration and place the cells into suspension. Schillinger and Ganon (1982) reported that 0.2 to

5.0% of the bacteria are retained by the screens. Faecal coliform bacteria concentrations are determined for each bottle and the degree of association with a particular size class is expressed as a percentage of total count.

Sedimentation rates for particulate matter is measured using established sediment trap techniques (cylindrical traps; aspect ratio 6:1; Bloesch and Burns, 1980; Blomqvist and Haqanson, 1981). Traps are placed at a depth 1m below the thermocline and are deployed for a period of 48 hours. The traps are cleaned and reset for incubation immediately following storm overflow events and harvested for particle size analysis. Sedimentation rates are measured for each of seven particle size classes: 0.45-1, 1-6, 6-10, 10-20, 20-53, 53-102 and >102 µm. Triplicate aliquots of the particle suspension collected from the traps are passed through individual screens (e.g., 102 µm) using a brief vacuum application at 15 psi and then through 0.45 µm filter. The screens are dried for a minimum of 3 h at 103-105°C and cooled in a desiccator prior to weighing. The mass of material passing through the 102 µm represents that associated with 0.45-102 µm fraction. The amount passing through the next smallest screens (53 µm) represents that associated with the 0.45 -53 µm fraction. Masses associated with individual size classes (e.g., 53-102 µm) are determined by subtraction. The smallest size (0.45-1 µm) includes solitary bacterial cells.

The sediment flux is calculated as the mass of material collected in the trap divided by the product of the trap area and the incubation period,

$$J = \frac{M}{At} \qquad (3.2)$$

where, A= area of the sediment trap opening (m^2)

J= sediment flux (g m^{-2} d^{-1})

M= mass of sediment collected in the trap (g)

t= time of incubation (d).

The sediment velocity (m d^{-1}) is calculated as the sediment flux divided by the water column suspended solids concentration,

$$v = \frac{J}{C_{ss}}$$ (3.3)

where, C_{ss}= water column suspended solids concentration (gm^{-3})

v = sedimentation velocity (m d^{-1})

The sedimentation loss rate coefficient (K_s) is calculated as the sedimentation velocity divided by the distance across which the particles must settle before they are lost to the bottom waters,

$$K_s = \frac{v}{H}$$ (3.4)

where, H= depth of the lake.

3.5.3 Measurement of Rate Coefficient for Death as Mediated by Irradiance

The irradiance mediated death rate coefficient K_i is estimated as follows:

$$K_i = \frac{\alpha I_0}{K_e H}[1 - \exp(-K_e H)]$$ (3.5)

where, α = proportionality constant which relates irradiance mediated death rate coefficient to irradiance over the entire depth (cm^2 cal^{-1}).

I_0 = average irradiance at the water surface (cal cm^{-2} day^{-1})

K_e= vertical light extinction coefficient in m^{-1}

H = depth of lake in m.

3.5.3.1 Measurement of proportionality constant α

To measure the proportionality constant α, in situ measurement of faecal coliform death rates and corresponding irradiance level are measured at different depths of the lake. Incubations are performed using dialysis tube, an approach which offers the advantage of exposing the bacteria to inlake conditions of irradiance, nutrients, pH, predation, salinity and temperature, while avoiding settling losses. Inocula are prepared as described before. Subsamples are collected for enumeration of initial faecal coliform bacteria concentrations and thirty-six, 100 ml aliquots of diluted waste water are placed in individual transparent dialysis membrane tubes (15.2 cm length, 2.74 cm inflated diameter, union carbide). Six tubes are attached by plastic clips to each of six weighted frames and the frames are deployed in the lake at depths of 0, 0.5, 1, 2, 3, and 8 m. A single tube is harvested daily from each depth for five days. an opaque trapulin is used during sample collection to prevent exposure of the tubes to direct sunlight. Samples are transported to the laboratory in a cooler at 4-8^{0}C and are analysed within 1-3 h of collection. Three dilution of each sample is made to insure statically acceptable plate count.

Incident irradiance is calculated from solar radiation measurements according to the method of Bannister . The daily average irradiance at different depth is calculated using Beer's law with measured values of daily average incident irradiance and the vertical attenuation coefficient (Thoman et al., 1987).

The value of α is calculated from the slope of death rate versus average daily irradiance curve.

3.5.3.2 Measurement of Vertical Light Extinction Coefficient

Measurement of total light penetration into a water body of water can be made by use of a pyreheliometer positioned in the boat or other suitable platform in the surface of water. The pyreheliometer measures the amount of total incoming solar radiation, usually in units of cal/cm^2 -min. Simultaneously an under water photometer is lowered and the radiation is recorded at each of a series of depths, throughout the water column. The concurrent measurements provide an estimate of the relative amount of radiation remaining at each

$$K_e = \frac{\ln(I / I_o)_2 - \ln(I / I_o)_1}{Z_1 - Z_2}$$
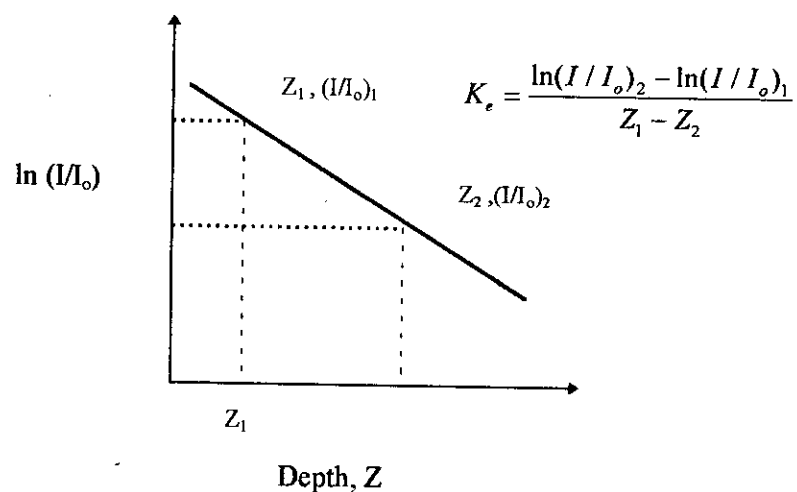
Fig. 3.4 Computation of Vertical Light Extinction Coefficient.

depth and the necessary data for computation of K_e as shown in the Fig. 3.4.

Another more simple method, but less accurate is to lower a target into the water until, by eye the target disappears. The depth at which the target just disappears can then be related to the extinction coefficient. A standardised target used in water quality work is the Secchi disk as shown in Fig. 3.5. Numerous measurements of "Secchi depth" have been made in many waterbodies. Sverdrup et al. and Beeton have developed empirical relationships between the Secchi depth z_s and the extinction coefficient (Thoman et al., 1987) as given by

$$K_e = (1.7-1.9)/z_s \qquad (3.6)$$
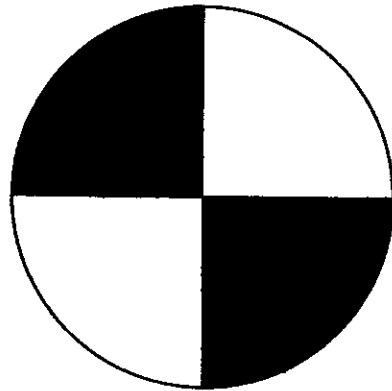
Fig. 3.5 Secchi disk

Di Toro (1978) has provided a theoretical and empirical basis for estimating the extinction coefficient as a function of non-volatile suspended solids (N), detritus (D), both in mg/l, and phytoplankton chlorophyll (P) in μg/l. For vertical sun angle, his estimate of K_e is

$$K_e = 0.025N + 0.174D + 0.031\,P \qquad (3.7)$$

The non-volatile suspended solids (the inorganic particulate) both absorb and scatter the light whereas the organic detritus and phytoplankton chlorophyll mainly absorb the light. Di Toro has shown that eq. 3.7 applies to K_e values of about less than 5.0 m^{-1}.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

Enteric pathogens enter water sources directly through human or animal contact or indirectly via sewage input or urban or rural runoff. Once in the water, various factors act to inhibit their survival. Primary among such factors are, temperature, irradiance, predation, nutrient deficiency, pH, sedimentation and biological extraction. However, the extent to which each of the above factors affects the decay of faecal coliform is still not completely known.

In this study an effort was made to determine the effect of various parameters on the death rate of faecal coliform. The following parameters were considered:

    i.   Water from different sources,

    ii. Temperature,

    iii. pH,

    iv. Nutrients.

The above parameters often vary in natural waters and the study is likely to lead to a better understanding of the decay mechanism of faecal coliform in natural water environment.

In addition, an attempt was made to assess the microbial self-purification capacity of natural water bodies and hence the loss rate of faecal coliform was determined in the following environment:

i. Actual stream environment,

ii. Actual lake environment.

4.2 DECAY RATE OF FAECAL COLIFORM IN DIFFERENT WATER SOURCES

Lakes and rivers are the two surface water bodies that are most frequently subjected to faecal pollution. So water from these two sources were used to examine the effect of sources of water on the decay of faecal coliform. River water was collected from Buriganga river, near China-Bangladesh friendship bridge. Lake water was collected from Dhanmondi lake. Samples were collected from a location at considerable distance from the bank. Samples were carried to the laboratories in a shrouded container to eliminate the effect of direct sunlight.

Samples were preserved in 300-ml bottles under room temperature. The room temperature varied from 26^0C to 28^0C. Faecal coliform concentration was measured daily using membrane filter technique. For each sample three enumeration were made each time and the mean was taken. The faecal coliform concentrations are expressed in colony forming unit (cfu) per 100 ml.

The decay of faecal coliform in the Buriganga river water was observed for 12 days (Fig.4.1). The initial concentration was 36000 cfu/100 ml. After 12 days the concentration reduced to 9 cfu/100 ml. The decay rate showed two distinct phase; the initial phase when the faecal coliform population reduced drastically at a rate of about 0.65 per day (Fig.4.2) was apparent until the fourth day following the collection of sample. There-after the decay rate
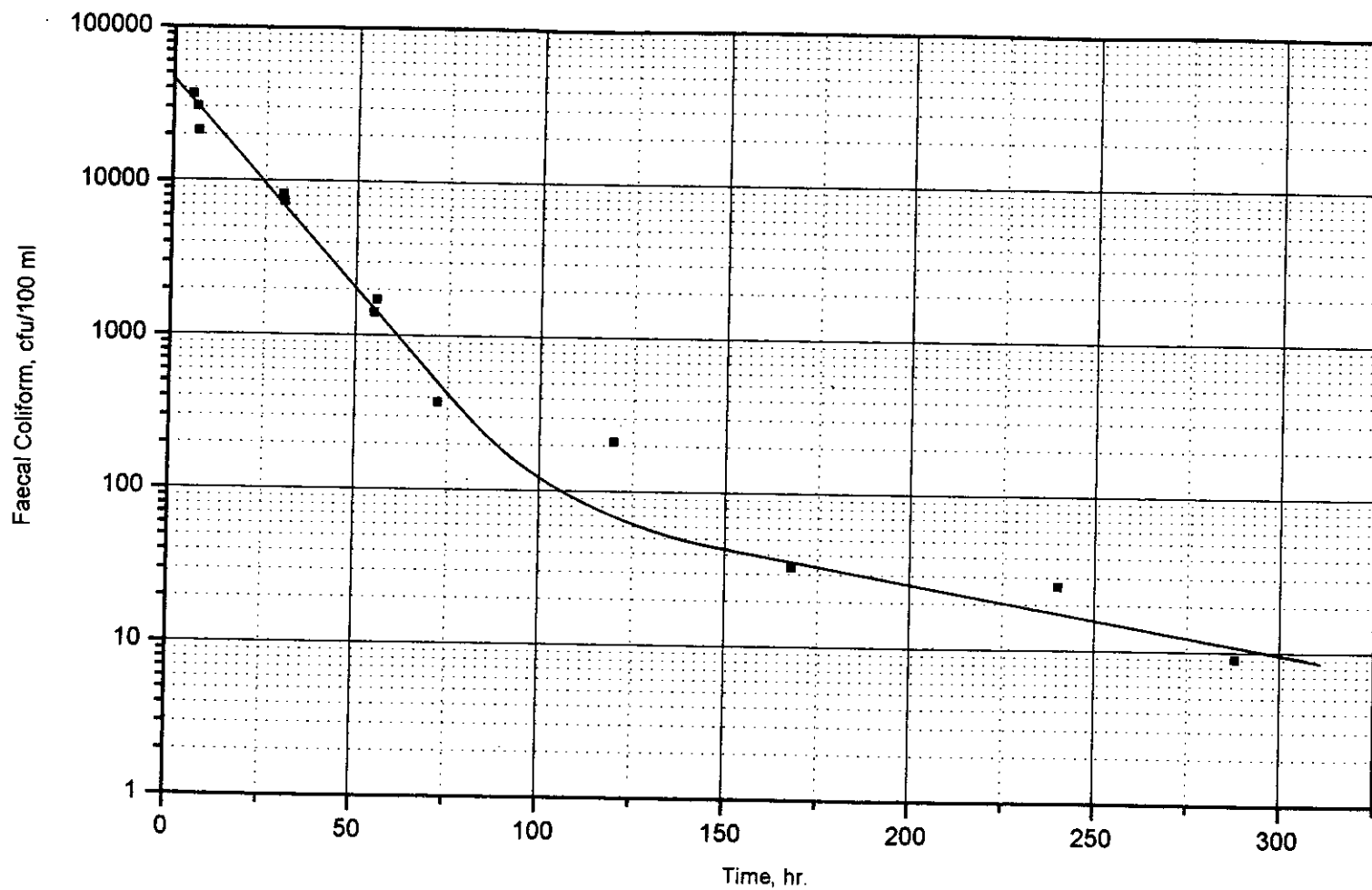
Fig. 4.1 Decay of faecal coliform in river water
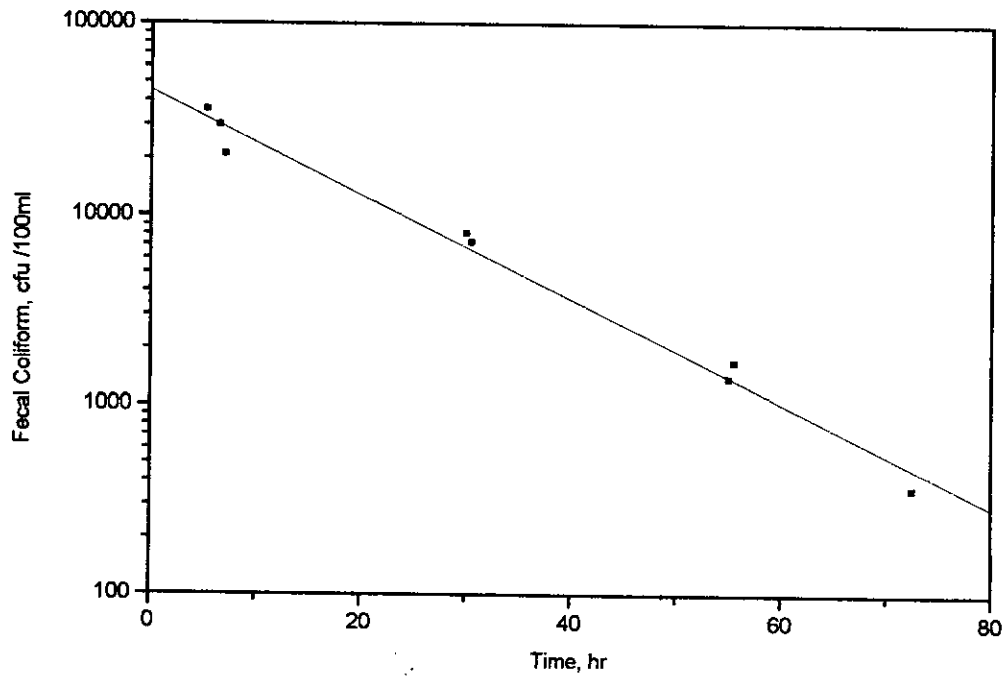
Fig. 4.2 Decay of faecal coliform in river water (initial phase)
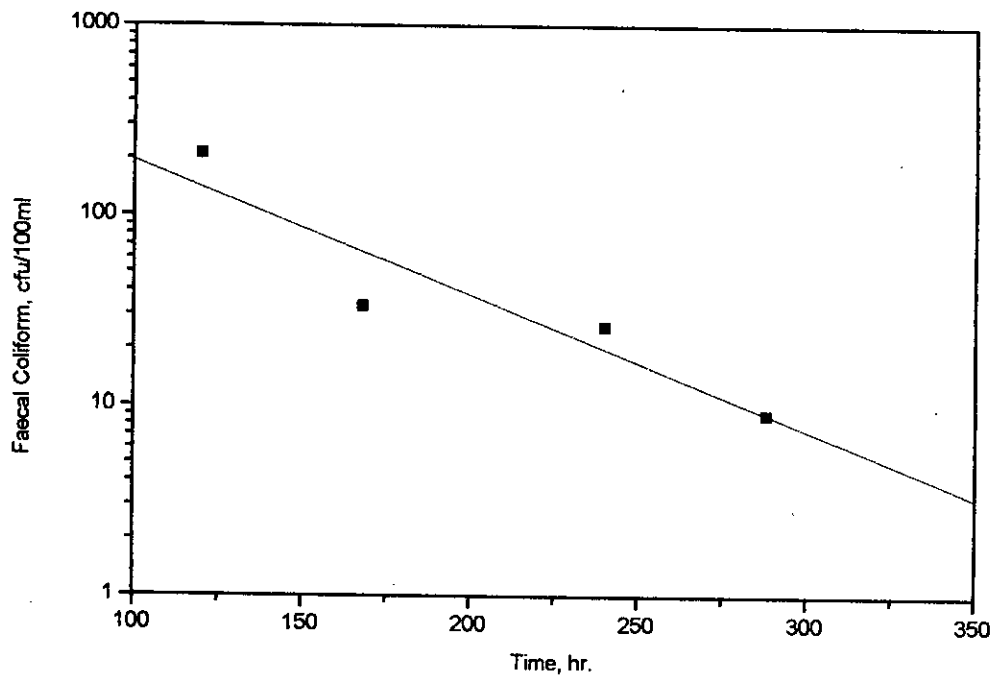
Fig. 4.3 Decay of more resistant portion of faecal coliform in river water

reduced significantly. This reduced decay pattern also showed a linear decrease when plotted in a semi-log paper. Hence the decay of faecal coliform in river water can be considered to be composed of two separate phases, one with a decay rate of K_1 applied to the initial population and the other with a decay rate of K_2 applied to a more resistant fraction. For the Buriganga river water the value of K_2 was found to be 0.17 day^{-1} (Fig.4.3) and the more resistant fraction was about 0.8% of the initial population.

The decay of faecal coliform in Dhanmondi lake water was observed for 10 days (Fig.4.4). During this period the concentration of faecal coliform reduced from 31000 cfu per 100 ml to 14 cfu per 100 ml. Like the decay pattern in river water the decay in lake water also showed two distinct phases. The decay rate during the initial phase was 0.64 day^{-1}. However, after this initial phase of rapid decay, the decay almost stopped and from the 5th day to the 10th day faecal coliform concentration reduced only 50%. For lake water the concentration of more resistant fraction of faecal coliform was found to be 0.27%.

For the purpose of comparison the decay rate of faecal coliform in distilled water was also observed (Fig.4.5). Samples were prepared by inoculating fresh sewage in sterilised distilled water. A similar pattern of a rapid decay followed by a very slow decay was also evident in this case. However, the initial decay rate (0.42 day^{-1}) was found to be much lower than that observed for lake and river water. The fraction of population that was more resistant to decay was only 0.1% of the initial population.

The decay rate and half-life of faecal coliform in different types of water are listed in Table 4.1.
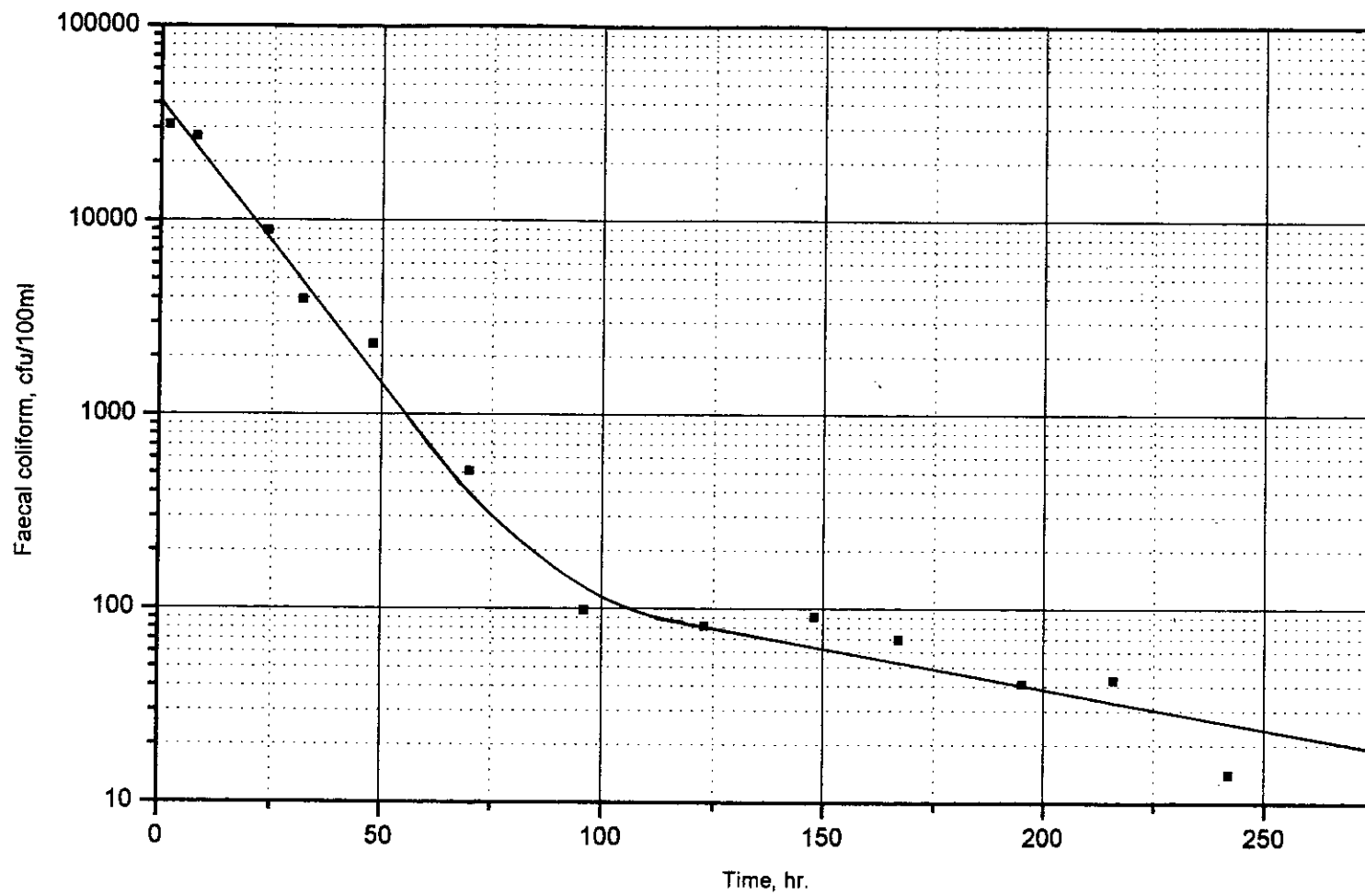
Fig. 4.4 Decay of faecal coliform in lake water
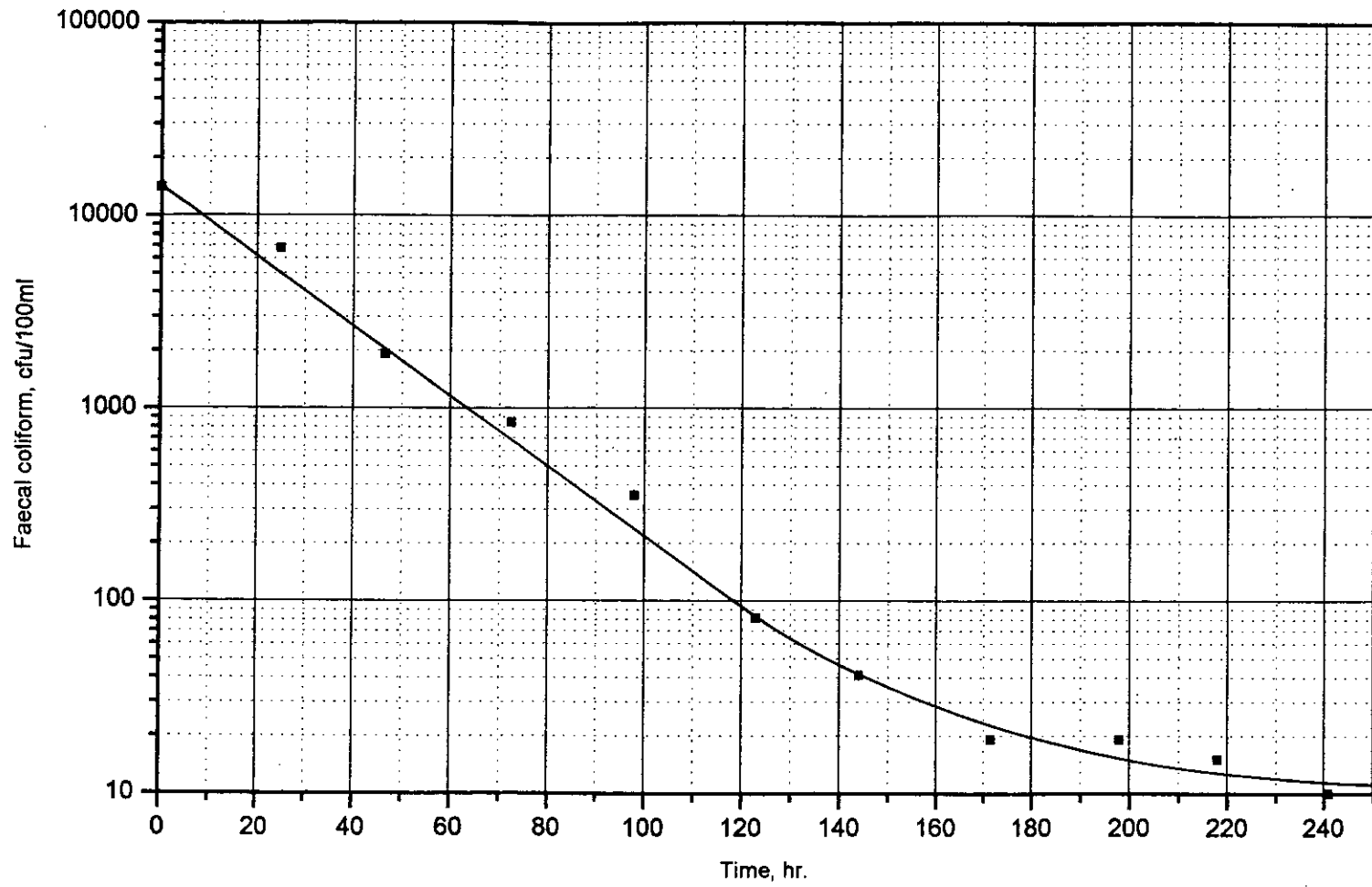
Fig. 4.5 Decay of faecal coliform in sterilised distilled water

Table 4.1 Decay rate and half-life of faecal coliform
in different types of water

Source of Water	Decay Rate (day^{-1})	Half-life (days)
River	0.65	0.46
Lake	0.64	0.47
Distilled water	0.42	0.72

It is apparent from the above results that

i. Faecal coliform decay rate is higher in natural waters and it survives longer in relatively good quality water.

ii. There is no significant difference in faecal coliform decay rate in river and lake waters.

iii. A resistant group of faecal coliform is present in natural waters which may persist in water for a very long period. The percentage of this resistant group is different in different type of water bodies.

The significantly higher decay rate in surface waters is probably the presence of toxic substances and trace metals in those waters which cause growth retardation and death. Another reason may be that during inoculation of faecal coliform in distilled water by sewage, significant amount of nutrients are introduced into the distilled water. The presence of nutrients may also retard the decay rate in distilled water. Moreover, some predators which are natural habitats of surface waters may also increase the death rate in river and lake water.

The higher percentage of faecal coliform in river water may be attributed to the fact that it take longer period for sewage to reach the river and in this period a sizeable fraction of the more susceptible fraction of faecal coliform dies. There is also a possibility that in river a small fraction of the faecal coliform may come from resuspension and most of this later group is possibly of the resistant type.

If we compare the survival of faecal coliform with the survival of some of the pathogens whose decay has been studied, we find that most pathogenic bacteria have a shorter survival period in aquatic environment than that found for faecal coliform group of bacteria. For example, time required for 100% infectivity loss for $V.$ $cholerae$ in river water at a temperature of 22°C is less than 0.75 day (Montgomery, 1985). However, some enteric viruses which are pathogenic to human, were found to survive in aquatic environment for longer period than does the faecal coliform. Polivirus can survive in river water for 29 to 35 days (Montgomery, 1985). Hence, it can be said that although faecal coliform is a satisfactory indicator of bacterial water quality, it is not an acceptable indicator for many pathogenic viruses.

4.3 EFFECT OF TEMPERATURE

Of the physical factors that affect the survival of faecal coliform in aquatic environment, temperature is probably the most important. To determine the effect of temperature on the decay of faecal coliform the decay rate was observed at five different temperatures

i.   below 4°C
ii. 20°C

iii. 27⁰C

iv. 32⁰C

v. 49⁰C

For each temperature two 300 ml samples were prepared by mixing fresh sewage with sterilised distilled water. Incubators were used to maintain a constant temperature. The decay rate below 4⁰C was observed by placing two samples in the refrigerator. Faecal coliform concentration of each sample was measured daily until a stable decay rate was obtained. The membrane filter technique was used for enumeration of faecal coliform. To minimise statistical error 3 dilutions of each sample were made and the mean value was taken. The decay rate at a temperature below 4⁰C was found to be very low with no definite pattern. Gradual increase in decay rate was observed from 20⁰C to 32⁰C. However, the decay rate increased sharply passing that range and at a temperature of 49°C all the faecal coliforms died within 6 hours. The decay rates and half-lives at different temperatures are listed in Table 4.2.

Table 4.2 Decay rate at different temperatures

Temperature ⁰C	Decay Rate (day^{-1})	Half-life (day)
20	0.255	1.18
27	0.42	0.72
32	0.59	0.51

It can be seen from the results that for the temperature range of 20°C to 32°C there is a strong correlation (variance=0.99) between decay rate and temperature (Fig. 4.7). This relation can be expressed by the equation

$$K_T = K_{20} \times 1.07^{(T-20)} \qquad\qquad (4.1)$$
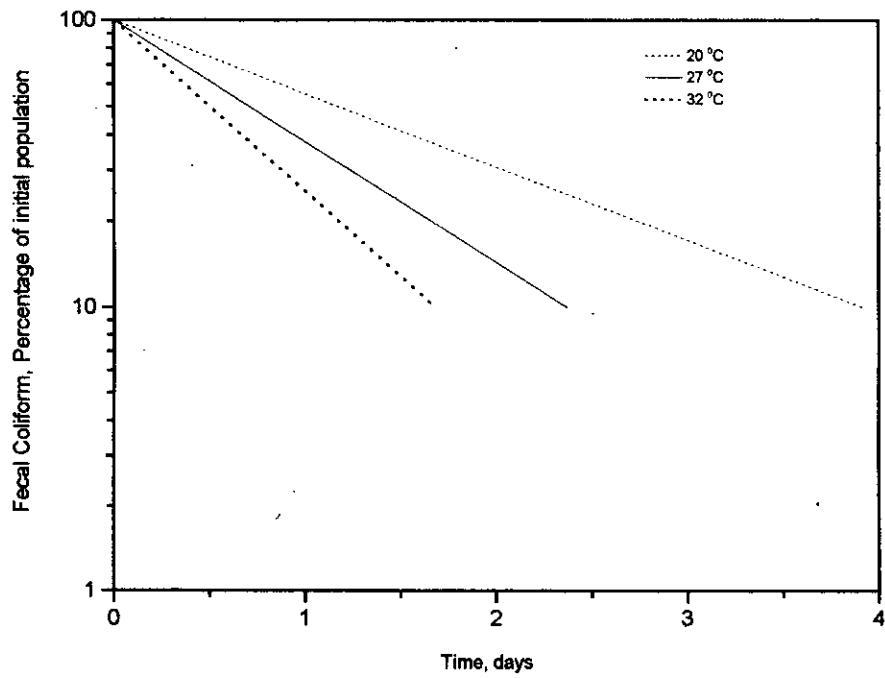
Fig. 4.6 Decay of faecal coliform at different temperatures
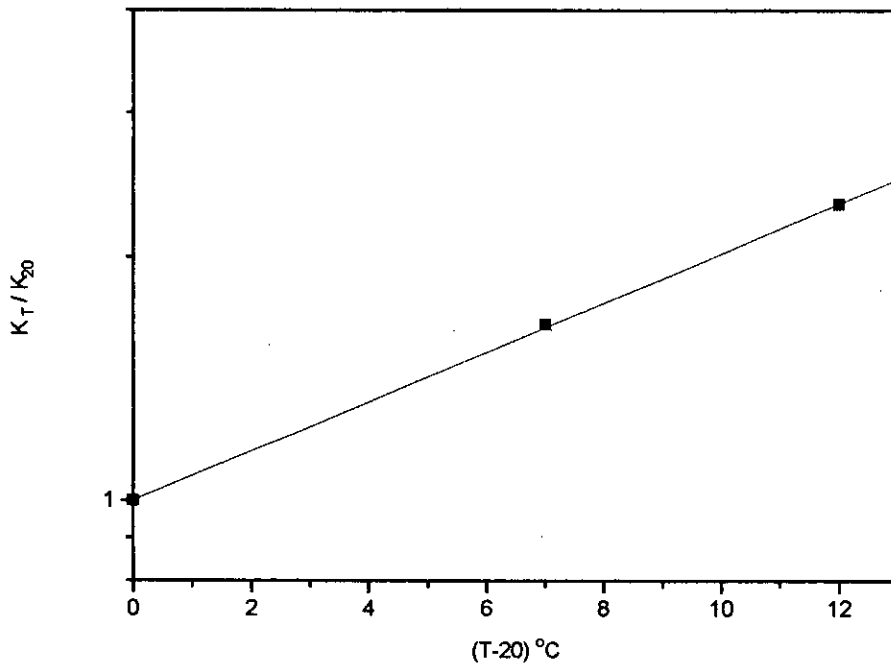
Fig. 4.7 Decay rate at different temperatures

where, K_T = decay rate at any temperature T^oC in day^{-1}, and

K_{20}= decay rate at 20^{o}C in day^{-1}.

The death rate of faecal coliform at low temperature is small because bacterial activities at low temperature is minimum. As a result most of the bacteria survives longer at low temperature. But as the temperature increases the rate of biochemical reaction that occurs within the cell also increases. Increased cell activities place added demand on nutrient reserves, which may not be renewed in dilute natural systems, leading to an increase in death rates.

Some researchers (Gaudy et al., 1990) have implicated two classes of molecules, lipids and proteins, to explain the effects of temperature on viability.

Lipids containing fatty acids are essential structural components of cell-membranes. At high temperatures, lipids may melt causing loss of the structural integrity of the membrane and leakage of the cell content. Damage to cell membrane also inhibits the release of enzyme to dissolve the organics and entry of food through cell walls for metabolism. Thus damage of cell membrane leads to the death of micro-organism.

The other class of molecules involved in effects of temperature on viability, i.e., proteins, perform much more varied functions in the cell than do lipids. Proteins, like lipids, are important structural components of membranes. Proteins are also structural components of the ribosome, along with RNA. Most of the many different protein molecules in the cell function as enzymes that catalyse the many reactions required for growth of the cell. For each of these roles, the specific protein involved must maintain a precise three

dimensional structure. Loss of function results from an alteration in the formation of the molecule. An increase in temperature affects proteins by causing thermal denaturation, an alteration of the functional spatial arrangement, which is usually irreversible. Elevated temperatures thus can inactivate many essential processes in the cell by inactivating the protein involved.

4.4 EFFECT OF pH

To determine the effect of pH on the decay of faecal coliform, the decay rate was observed at five pH level:

    i. pH 4.0

    ii. pH 6.0

    iii. pH 7.0

    iv. pH 8.0

    v. pH 9.0

The samples were prepared by mixing fresh sewage with 300 ml of sterilised distilled water. To maintain constant pH, buffers were used, which resist radical changes in the hydrogen ion concentration when acid or alkali is produced in the medium. Death rates were calculated over periods when the population was clearly in decline to avoid interference from after-growth effects in the early stage of incubation. Lowest decay rate was observed at a pH level of 7.0. Deviation from the neutral condition prompted an increase in faecal coliform decay. However, small changes in pH (from 6.0 to 8.0) caused minor change in decay rate. In the strong alkaline environment (pH=9.0) the decay rate is about 33% higher than that at neutral condition (Fig.4.8). A

sharp increase in decay rate (180% higher than that at pH 7.0) was observed at strong acidic environment (pH=4.0)

An interesting observation was that, the after-growth effect is very prominent at a pH of 7.0. But as pH varied from neutrality the after-growth was also reduced. No after-growth was observed at pH 4.0.

Table 4.3 Decay rate at different pH level

pH	Decay Rate (day^{-1})	Half-life (day)
4.0	1.09	0.276
6.0	0.402	0.748
7.0	0.39	0.763
8.0	0.417	0.72
9.0	0.52	0.578

Since micro-organisms are capable of altering the internal pH of the cell, the mechanism by which they are affected by the pH of the aqueous environment is not yet completely understood. An obvious explanation might lie in the effect of pH on enzymatic activity, since each enzyme is active within only a specific and usually narrow pH range and displays maximum activity at an optimum pH.

Another important factor may be the effect of pH on the transport of materials across the membrane. The effects of pH on transport are both direct and indirect. A direct effect is exerted through alteration of the pH gradient across the membrane. As pH gradient is the driving force behind transport of materials into the cell, any alteration of this force affects the viability of the cell. For compounds that are transported by binding to specific membrane
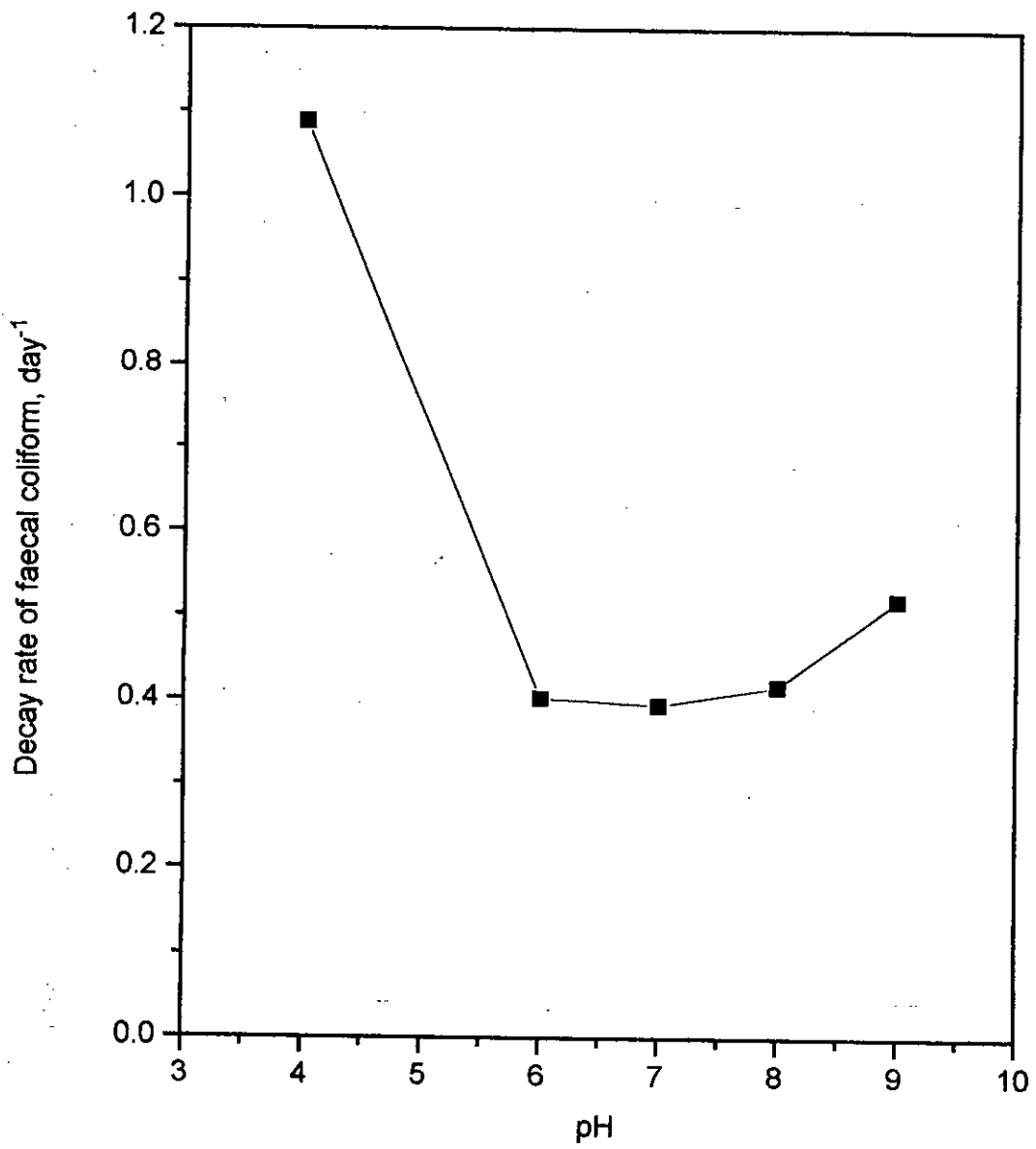
Fig. 4.8 Decay rate of faecal coliform at different pH level

proteins, pH may control the configuration and thus the activity of the binding protein.

pH of the aqueous environment also determines the ionisation state of the nutrients required by the cell and of the compounds that may be toxic to it. Cells are more permeable to nonionised than to ionised compounds; hence, ionisation of a required nutrient may make it unavailable to the cell. This explains why the aftergrowth effect was not observed when the environment became strongly acidic or alkaline.

An interesting factor about the effect of pH on the decay of faecal coliform is that although faecal coliform can not survive well in strongly acidic environment, some human pathogens, e.g., *salmonella* and *shigella* are capable of growth over a broad range of pH (pH 4.0 to pH 9.0). Hence, in strongly acidic aquatic environment, which may occur where streams receive acidic industrial waste, despite the absence of faecal coliform, some human pathogens may be present.

4.5 EFFECT OF NUTRIENTS

The nutrients that are important for the growth and survival of micro-organisms in aquatic environment are carbon, nitrogen and phosphorous. In most cases however, nitrogen and phosphorous are the ones that limit growth. Nitrogen is available in three forms-- ammonia (NH_4), nitrate (NO_3) and nitrite (NO_2). Phosphorous is mainly available as phosphate (PO_4). To investigate the effect of nutrients on the survival of faecal coliform, its decay was observed at three different levels of nutrients. Table 4.2 shows the concentrations of different forms of nutrients at these three levels.

Table 4.4 Nutrient concentrations of different levels.

Nutrients	Level 1	Level 2	Level 3
Ammonia, NH_4 (mg/l)	0.2	0.6	2.5
Nitrate, NO_3 (mg/l)	0.39	2.1	6.8
Nitrite, NO_2 (mg/l)	0.31	2.2	7.2
Phosphate, PO_4 (mg/l)	0.59	0.9	1.3

The decay rate at nutrient level 1 was found to be 0.54 per day. As the nutrient level increased, survival of faecal coliform also started increasing. However, at nutrient level 2, no after-growth was observed. But at a high concentration of nutrients, the faecal coliform population increased until the third day. Thereafter the population started decreasing again in exponential form.

From Fig. 4.9, it can be clearly seen that as nutrient level increases the decay of faecal coliform in aquatic environment diminishes. Two reasons may be cited for this. The net decay rate is the sum of growth and death rates of micro-organisms in definite media. In an unfavourable environment, the growth rate is zero and decay rate equals the death rate. A nutrient reach environment is favourable for bacterial growth and in such environment the aftergrowth of faecal coliform occurs which reduce the net decay rate. Secondly, as nutrient level increases the grazing on faecal coliform by predators decreases resulting in a further decrease in decay rate.
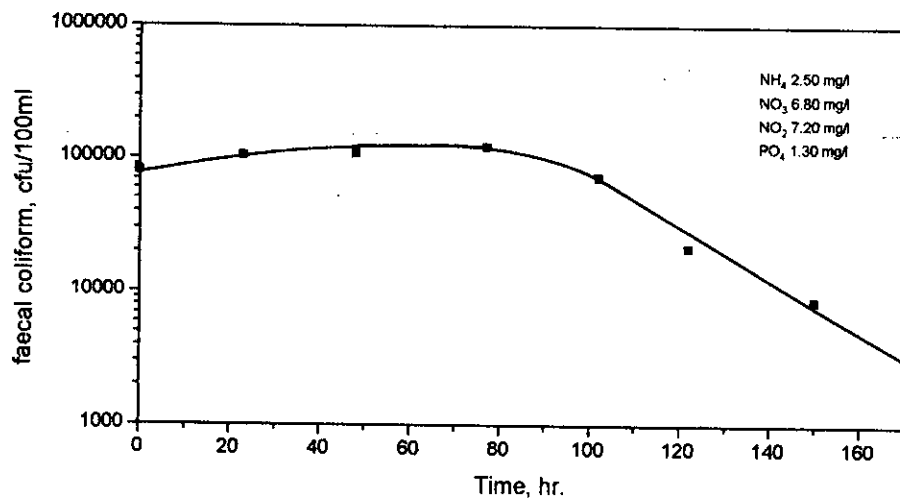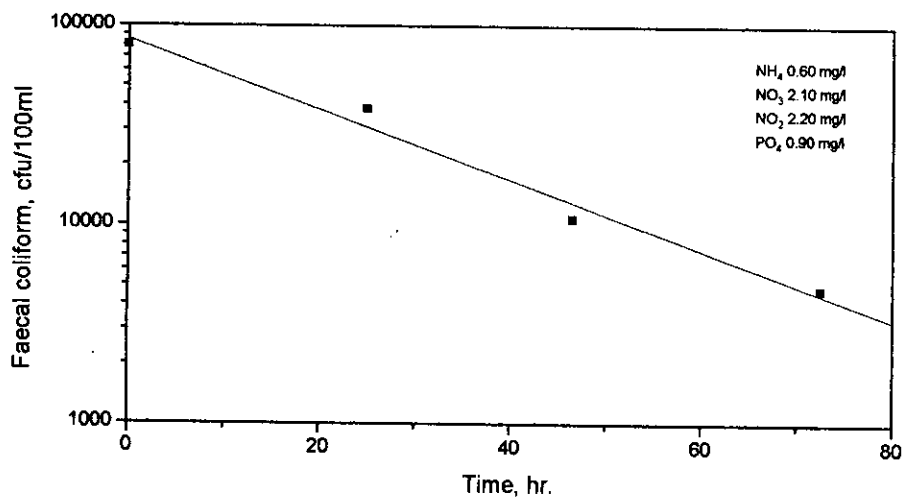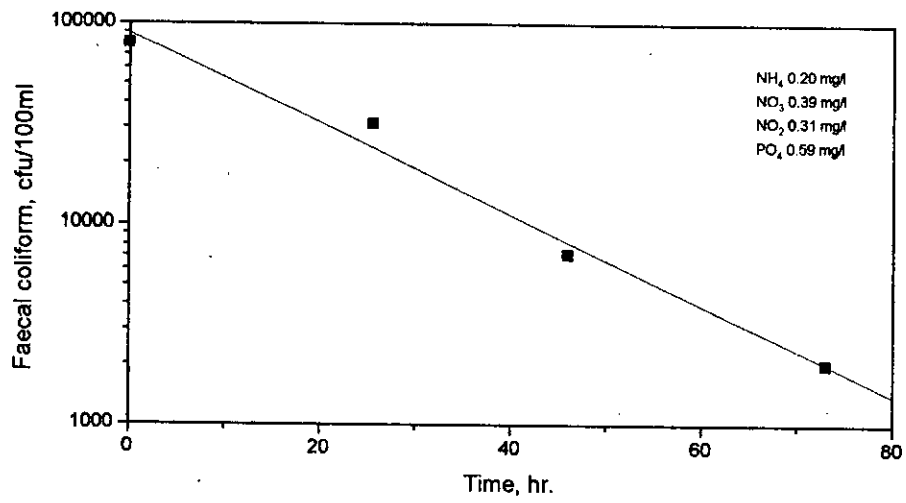
Fig. 4.9 Decay of faecal coliform at different nutrient levels

4.6 LOSS RATE OF FAECAL COLIFORM IN DHANMONDI LAKE

Direct measurement of decay rate of faecal coliform in actual lake environment requires temporary suspension of all inflow and outflow. As it was not possible, the three components of loss rate of faecal coliform in Dhanmondi lake were measured separately to obtain the overall decay rate. The three major components of decay in lake environment are:

i. Rate coefficient for death in the dark including the effects of temperature, salinity, predation etc. (day^{-1})

ii. Rate coefficient for death as mediated by irradiance (day^{-1})

iii. Rate coefficient for sedimentation loss (day^{-1})

i. Rate coefficient for death in the dark: Water samples were collected from the centre of the lake in a sterilised 300 ml bottle. It was carried to the laboratory in a shrouded container to avoid exposure of the sample to direct sunlight. The sample bottle was covered with aluminium foil and was kept in room temperature. Faecal coliform count was made daily using membrane filter technique. Three observations were made for each enumeration and the mean was taken to ensure statically acceptable count. The death rate in the dark was found to be 0.41 day^{-1} (Fig. 4.10). This rate reflects the combined effect of temperature, salinity, predation etc.

ii. Rate coefficient for death as mediated by irradiance: The irradiance mediated death rate coefficient representative of conditions over the entire depth of lake is

$$K_i = \alpha I_{avg.}$$ (4.2)

where, α (cm^2 cal^{-1}) is the proportionality constant which relates irradiance mediated death rate coefficient to irradiance over the entire depth.
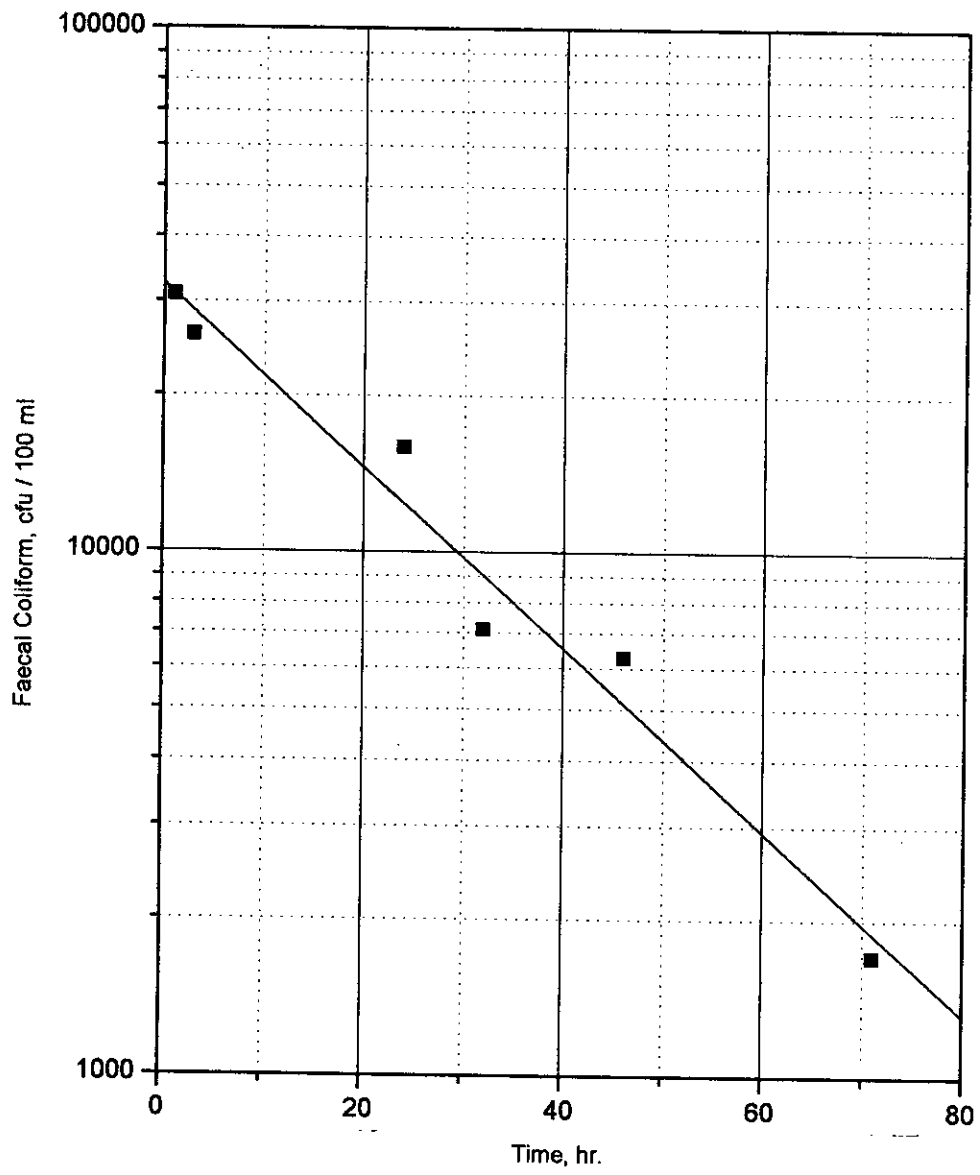
Fig. 4.10 Decay of faecal coliform in the dark in lake water

$$I_{avg} = \frac{I_0}{K_e H}\left[1 - \exp(-K_e H)\right]$$

(4.3)

where, I_0 = average irradiance at the water surface (cal cm^{-2} day^{-1})

K_e = vertical light extinction coefficient in m^{-1}

H = depth of lake in m.

The value of α was studied by many researchers, including Manchini, et al (1978), and Auer, et al (1992); The range of α varies between 0.004 to 0.009. For tropical and eutrophic urban lake similar to Dhanmondi lake α can be taken as 0.005.

The value of I_0 was collected from the Department of Meteorology. The maximum value of 385 cal^{-1}cm^2 day^{-1} for I_0 was found during the month of April. The minimum value was found to be 215 cal^{-1}cm^2 day^{-1} in January.

The light extinction coefficient K_e was measured using Secchi disk. The depth up to which Secchi disk was visible in the lake water varied between 0.51 m to 1.1 m. For these two values the light extinction coefficient varied from 1.545 m^{-1} to 3.725 m^{-1}.

The average depth of Dhanmondi lake varies between 2.5 m to 3.2 m. So the irradiance mediated death rate coefficient of Dhanmondi lake, determined by using equation 3.5. varies between 0.04 per day and 0.209 per day.

iii. __Rate Coefficient For Sedimentation Loss__: The loss rate due to sedimentation can be estimated by the following equation:

$$K_s = \frac{v_s}{H}$$

(4.4)

where, v_s = sedimentation rate in m/day and

H = depth of lake in m.

However, the sedimentation rate in Dhanmondi lake could not be measured because of unavailability of sedimentation traps. Auer et. al. (1992) determined the sedimentation rate in Onondoga lake -- a tropical, eutrophic, urban lake in syracuse, New York, and found out a value of 0.68 m/day. If this value is used then the loss rate due to sedimentation in Dhanmondi lake, determined by using equation 3.4, varies between 0.21 day^{-1} to 0.27 day^{-1}.

Total decay rate: Summing up the three components of loss rate of faecal coliform in Dhanmondi lake, we find that it varies between 0.66 day^{-1} to 0.89 day^{-1}. The contribution of the different components to the overall loss rate of faecal coliform in Dhanmondi lake is shown in Table 4.5.

Table 4.5 Contribution of different components to the total loss rate in Dhanmondi lake

Component of decay	Rate (day^{-1})	Percentage of total loss rate
Rate coefficient for death in the dark	0.41	62 - 46
Rate coefficient for death as mediated by irradiance	0.04 - 0.21	6 - 24
Rate coefficient for sedimentation loss	0.21 - 0.27	32 - 30
total loss rate	0.61 - 0.89	

It is apparent from the above results that apart from the natural death rate, sedimentation has a major contribution on the total loss rate of faecal coliform in the actual lake environment. The impact of the solar radiation varies with the turbidity of the lake water. When the turbidity is high, sunlight can not penetrate much into the water column resulting in a low death rate. On the other hand, when the water is comparatively clear, the decay rate due to sunlight also becomes significant. Comparing the loss rate of faecal coliform under laboratory condition to that obtained in the dark, it can be said that irradiance in the visible range has a marked impact on the survival of faecal coliform.

4.7 LOSS RATE OF FAECAL COLIFORM IN THE RIVER BURIGANGA

4.7.1 Pollution load

The river Buriganga, a tributary of river Dhaleswary, flows by the south western periphery of the densely populated area of the city of Dhaka. The river Turag demarking the western boundary of Dhaka city falls in the river Buriganga. The upstream of the river Buriganga above the confluence of the river Turag has silted up over the years to the extent that in the dry season during March and April, this part of the river becomes dry. As a result, during this lean period of the year, the discharge of the river Turag is the only source of water of the river Buriganga.

Dhaka is a fast growing city in Asia-Pacific region with a present population of about 9 million. The population of this city is expected to be over 10 million by the end of this century. Waste management in the city did not receive due importance in the past. The only existing sewage treatment plant

is already overloaded receiving only 15 percent of the total sewage produced in the city. Industries and commercial utilities has grown up without effective waste management facilities. As a result the river Buriganga directly or indirectly receives a large amount of sewage polluted surface runoff, untreated industrial effluents and partially treated sewage effluents. As there is no strict law prohibiting direct discharge of sewage and waste into the river, there are a large number of outlets pouring out pollution load directly into the river. So, an extensive river survey was undertaken to find suitable reach for sampling.

4.7.2 River Survey

A number of field visits were made along the Buriganga river both on feet and by boat to gain first hand knowledge about the existing sources of faecal pollution. It was observed that after the construction of the flood protection embankment the waste discharge into the river is now regulated to a great extent to point sources.

The river Buriganga receives huge pollution load via the river Turag from the pumping station at Kallayanpur. Surface water runoff from the adjacent low-lying area accumulates in a reservoir on the city side of the embankment. This water is then pumped towards the riverside by a high capacity pump. As there are many open latrines in the catchment area of the khals that discharge into the reservoir, the surface water runoff carries a huge amount of microbial load. At Hazaribagh, tannery and domestic wastes are currently being impounded behind the Dhaka city flood protection embankment. There is a manually operated sluice gate for passage of wastes towards the riverside. however the flow through this sluice was found to be very small during the time of survey (February- April). A considerable human settlement was

observed on the riverside of the embankment and they contribute a large amount of microbial load to the river. At Chadnighat there is a water treatment plant; the water is withdrawn from the river and then treated to be used for human consumption. In the downstream from the treatment plant indiscriminate disposal of solid waste was observed, especially at Farashganj and Babubazar. A number of outlets pouring sewage was also observed. At Sadarghat IWTA terminal direct dumping of human faeces was observed. Another source of microbial pollution is the steamers and launches plying in the river, which dumps faeces into the river. A large amount of pollution load is contributed by Dholai Khal, which discharges into the river near Farashganj. Sewage outlet was also observed near Postagola.

At Pagla the river receives effluents from the only existing sewage treatment plant of the city. Despite a comparatively lengthy detention period of 7 days, considerable microbial load was found in the effluent (1.1×10^9 faecal coliforms per day). No apparent major source of faecal coliform was observed downstream of the treatment plant.

Table 4.6 Faecal coliform concentration of some selected locations in the Buriganga river.

Location	Faecal colifrom concentration, MPN/100ml
Kallayanpur	12,000-14,000
Hazaribagh	11,000-15,000
Chadnighat	16,000-19,000
China-Bangladesh Friendship Bridge	30,000-36000
Downstream of Sewage Treatment Plant	27,000-30,000

4.7.3 Sampling

Since there is no distinct major point source of faecal coliform downstream of Pagla Sewage Treatment Plant (STP), the downstream reach from STP to the Dhaleswari river was selected as study reach to determine the loss rate of faecal coliform in the Buriganga river. Three sampling locations were selected, each 2 km apart. The first upstream location was chosen about 1 km downstream of the Pagla sewage treatment outfall to avoid interference of the effluent from the treatment plant. The sampling stations are shown in Fig. 4.11.

At each sampling station 4 samples were collected within a period of 12 hours at an interval of about 3 hours. The samples were collected in a 300-ml sterilised BOD-bottle from the centre of the river. The bottles were kept in ice-box to minimise decay during transportation. Each sample was tested within 2 hours of collection. For each samples 3 dilutions were made and the mean value was taken. The average of the four values taken at each station was used as the mean tidal concentration at that station.

Table 4.7 Average concentration of faecal coliform at the sampling site over the tidal period

Sampling Station	Mean tidal concentration of faecal coliform, cfu/100ml
1	29,000
2	10,500
3	9000
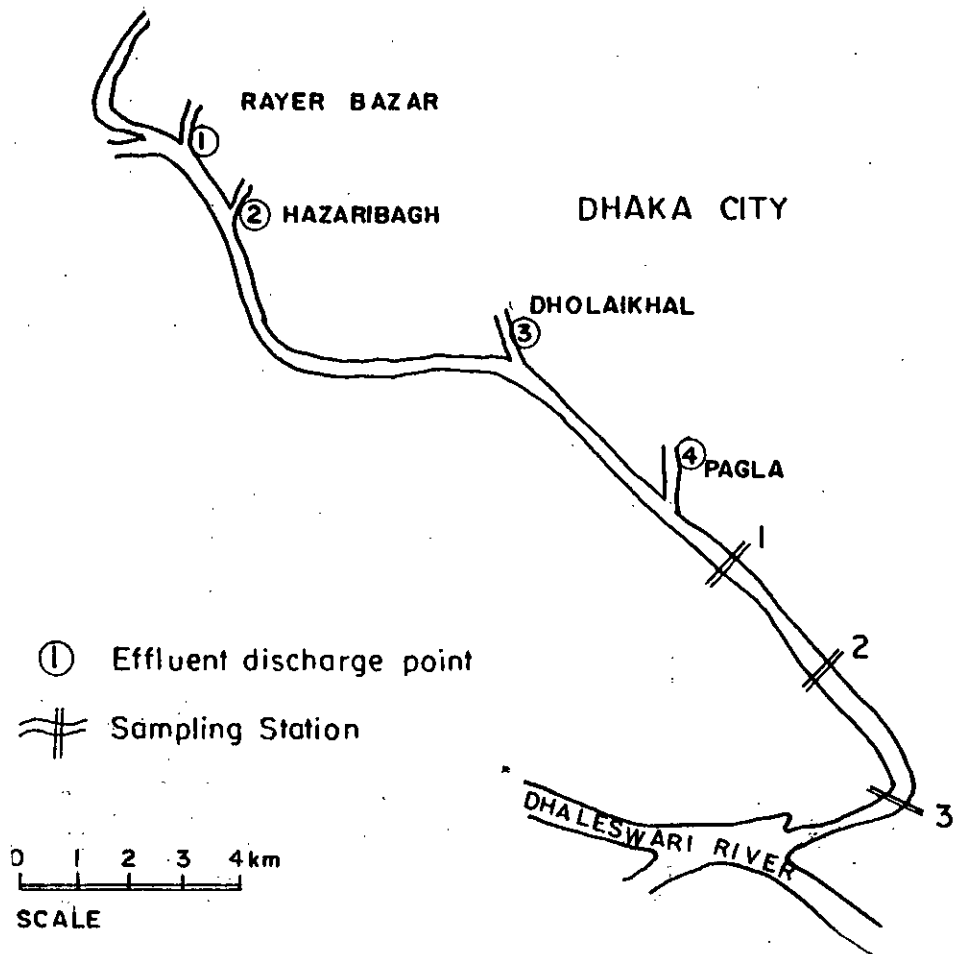
Fig. 4.11 Effluent discharge points and sampling stations in the Buriganga river

4.7.4 Time of Passage

For calculation of travel time from one cross-section to another along the river Buriganga, the steady state Lagrangian concept was adopted. In this concept time of passage is taken as occupied channel volume displaced in blocklike manner by the tributary streamflow: $t=CV/Q$, where t is the time of passage in second, CV is the occupied channel volume in m^3, and Q is the tributary flow in m^3/second.

As tidal effect is prominent in the river, hence net flow over a tidal cycle was considered instead of the instantaneous flow. Also, the occupied channel volume was taken as the one during mean-tide.

In situ flow measurement could not be made. Hence the hydrodynamic model developed by Institute of Flood Control and Drainage Research (IFCDR) was used to generate the flow data. The water level at six boundary locations were obtained from the Department of Hydrology. The river was divided into five segments and the cross-sectional profile at the boundaries were collected from the Surface Water Modelling Centre (SWMC). For calculation of occupied channel volume of each segment, the average of the cross-sectional area during mean tide at the two boundary was considered. Table 4.8 shows the time of passage data from 0 km to 17 km of the river Buriganga for the day of sample collection.

Table 4.8 Calculation of time of passage

Distance from 0 km, km	Cross-sectional area at mean tide, m^2	Mean tidal flow, m^3/s	Time of passage from 0 km, hr.
0.5	1354	64	2.94
4	1521	64	24.79
6.5	1853	64	43.11
11	1169	64	72.63
14	1132	64	87.62
17	595	64	98.86

4.7.5 Loss Rate

The average faecal coliform concentration was converted to faecal coliform load using the formula: W=QC, where W is the faecal pollution load in cfu/day, Q is the net flow in m^3/day and C is the concentration of faecal coliform in cfu/m^3. Then W was plotted against time of passage in a semi-log paper. The slope of the curve provided the decay rate in actual stream condition. For Buriganga river it was found to be 0.716 per day.

Table 4.9 Travel time and pollution load for the sampling stations

Sampling Station	Travel Time, (hr.)	Total Number of Faecal Coliform $\times 10^{15}$ per day
1	0	16.03584
2	10	5.80608
3	17.5	4.97664
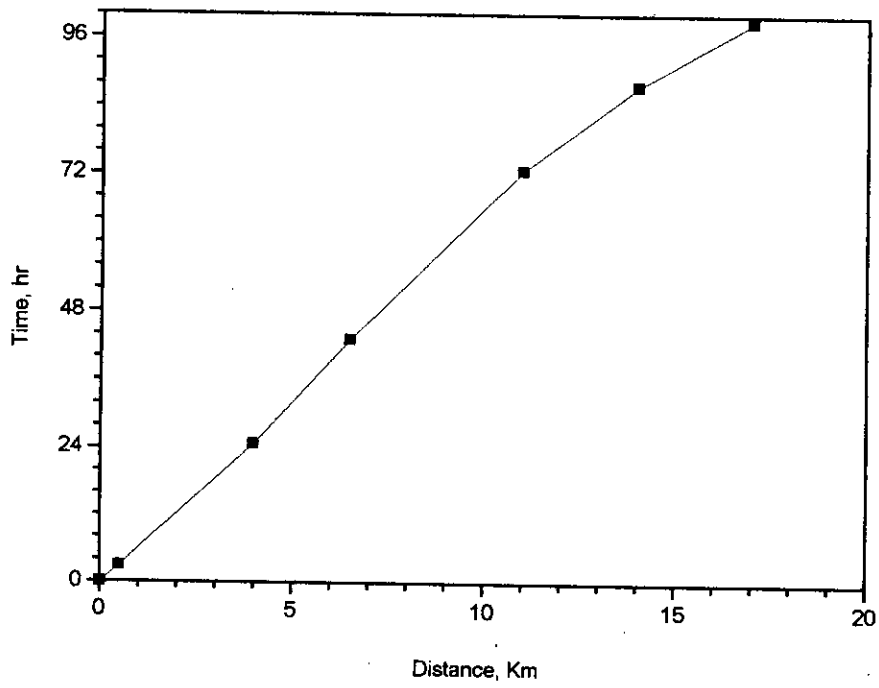
Fig. 4.12 Travel time for the Buriganga river
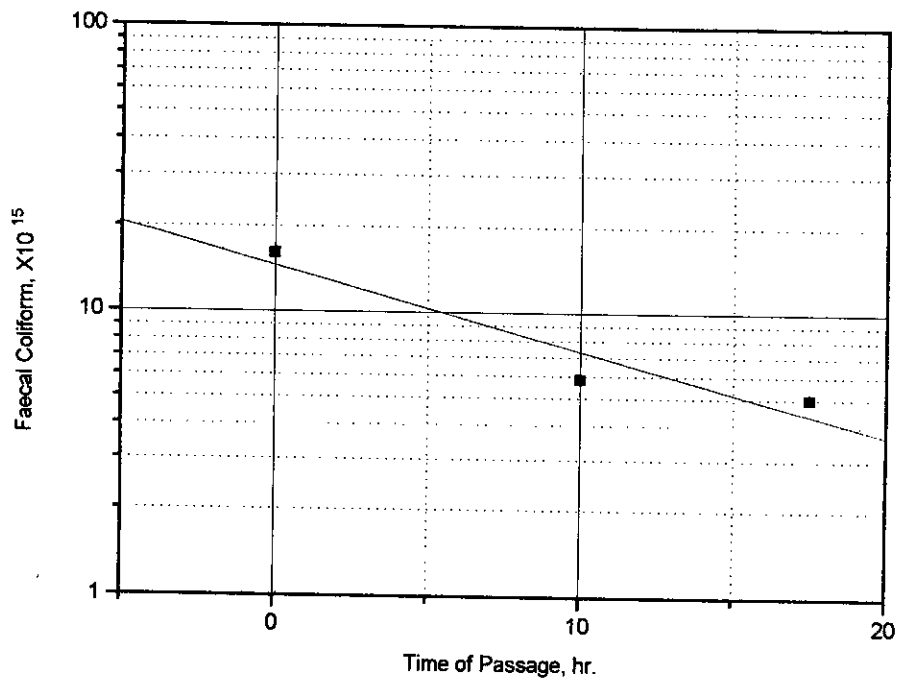
Fig. 4.13 Decay of faecal coliform in the Buriganga river

The decay rate in actual stream environment reflects the effect of a number of different factors. The major among those are -- predation, sedimentation, resuspension, biological extraction, nutrient deficiency, effect of toxic substances, effect of sunlight, temperature and pH. The decay rate of faecal coliform in the Buriganga river water obtained in the laboratory represents the effect of predation, nutrient deficiency, effect of toxic substances, temperature, pH and the effect of irradiance in the visible range. Hence the difference between this two decay rate should provide us the numerical estimate of the combined effect of sedimentation, resuspension, biological extraction and irradiance in the ultra-violet range. However, we find that the difference between these two values is very small. The decay rate in actual stream environment (0.716 per day) is only 10% higher than that in the laboratory. Hence it can be concluded that the combined effect of sedimentation, resuspension, biological extraction and the irradiance in the ultraviolet range on the survival of faecal coliform is very small. The reason behind this is probably that, sedimentation and resuspension in the Buriganga river usually balances one another. And as the river is tidal, turbulence is high, so the opportunity for biological extraction is small. High turbidity of the river water is probably the reason that sunlight in the ultraviolet range has little effect on the survival of faecal coliform in actual stream environment. Because of the high turbidity, ultraviolet ray can not penetrate much into the water column. Moreover, the ultra-violet ray constitutes only 3% of the total sunlight and hence its effect on bacterial decay is not significant. However it should be mentioned here that there could be some unaccounted for source of faecal pollution within this reach which could have reduced the decay rate in actual stream environment.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The objective of this research was to provide a better understanding of the complex processes that govern the microbial self-purification in aquatic environment. Faecal coliform group of bacteria was used as an indicator of microbial water quality. In this study the decay of faecal coliform was observed under various laboratory conditions and also in actual stream and lake environment. Information regarding the survival of faecal coliform under various environmental conditions provided valuable insight into the effect of different environmental parameters on the survival of faecal coliform and also on the applicability of faecal coliform bacteria as an indicator organism under the above conditions.

The following conclusions can be drawn from this research work:

- The decay of faecal coliform in lake water and river—water under laboratory conditions can be considered to be composed of two phases; an initial phase of rapid decay which is then followed by a very slow decay rate. For the Buriganga river water, the initial decay rate K_1 was 0.65 per day and that for Dhanmondi lake water was found to be 0.64 per day. The decay rate in the second phase (K_2) for river water was 0.17 per day and that for lake water was 0.102 per day.

- The decay rate of faecal coliform in both river water and lake water is significantly higher than that in sterilised distilled water. The presence of

toxic substances and trace metals and predators in surface water bodies may be responsible for this higher decay rate in natural waters.

- Temperature has a significant influence on the decay of faecal coliform in aquatic environment. At very low temperature faecal coliform population remains almost unchanged for a long period. But as the temperature increases the decay rate also starts increasing and at about 50°C faecal coliform cannot survive for more than 6 hours. For the usual range of variation of temperature in our country the relationship between temperature and decay rate can be expressed by the equation

$$K_T = K_{20} \times 1.07^{(T-20)} \tag{5.1}$$

where, K_T = decay rate at any temperature T°C in day^{-1} and

K_{20} = decay rate at 20°C in day^{-1}.

- pH of the aquatic environment also significantly affects the survival of faecal coliform. Decay rate is minimum at neutral condition (pH=7.0). However, within a pH range of 6 to 8, there is little variation in decay rate. But in strongly alkaline condition (pH=9.0), the decay rate is about 33% higher than that at neutral condition. And in strongly acidic environment (pH=4.0), faecal coliform can not survive for a period more than three days.

- The presence of nutrients usually retard the decay of faecal coliform in aquatic environment. Growth of faecal coliform has been observed in highly nutrient rich water, but the nutrient concentration of the surface water bodies rarely reaches that level to promote after-growth.

- In aquatic environment faecal coliform survives longer than most of the pathogenic bacteria whose decay under similar conditions is known. But some pathogenic viruses, e.g., polivirus, survive for a longer period in aquatic environment than does the faecal coliform. Hence, although faecal coliform is a good indicator for bacterial water quality, it should not be used as an indicator of pathogenic viruses. Similarly at a strongly acidic environment faecal coliform cannot serve as a good indicator of faecal contamination as some of the human pathogens are known to survive well within a broad pH range (4.0 to 9.5).

- The loss rate of faecal coliform in the Dhanmondi lake varies between 0.66 per day to 0.89 per day. Sedimentation was found to have a major effect on the loss of faecal coliform in lake environment.

- The loss rate of faecal coliform in the Buriganga river was found to be 0.716 per day. This is only 10% higher than that obtained in laboratory. From this, it can be concluded that the combined effect of sedimentation, resuspension, biological extraction and irradiance in the ultraviolet range on the loss of faecal coliform is very small.

5.2 RECOMMENDATIONS

- The utility of the faecal coliform bacteria as an indicator of the presence of pathogenic and other faecal organisms depends to a degree on the survival of faecal coliform and other organisms under similar environmental conditions. So a study of various pathogens under similar conditions may be undertaken, which would provide the justification of using faecal coliform as an indicator organism.

- The decay of faecal coliform in different streams of Bangladesh should be studied. This would provide valuable information regarding the effect of morphological characters of rivers on the loss rate of faecal coliform.

- The decay and survival of other indicator organisms, e.g., *faecal streptococci, E. Coli.* under various environmental conditions should be studied.

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APPENDIX A

Decay of Faecal Coliform at Different Temperatures and pH
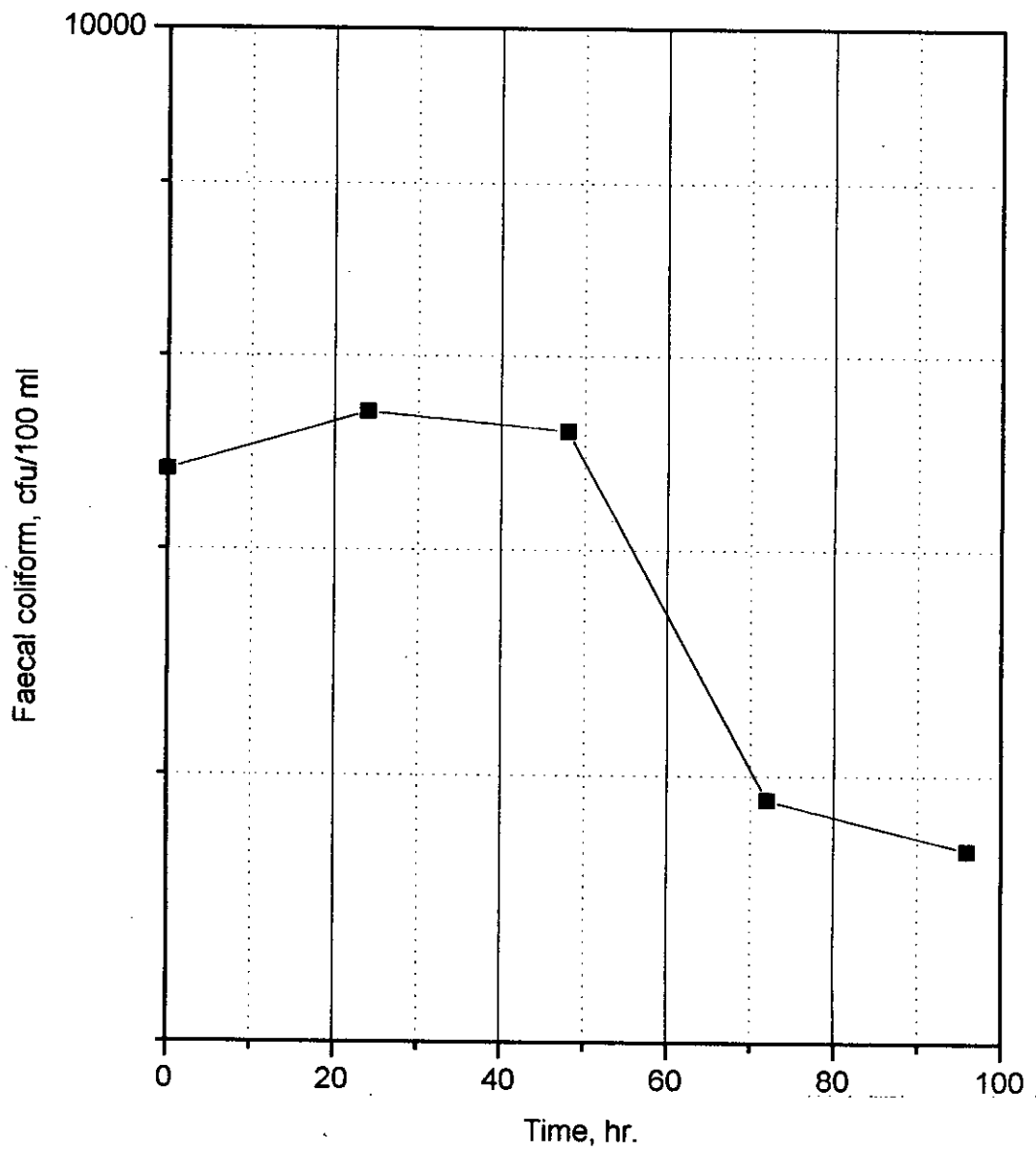
Fig. A.1 Decay of faecal coliform below 4^0 C
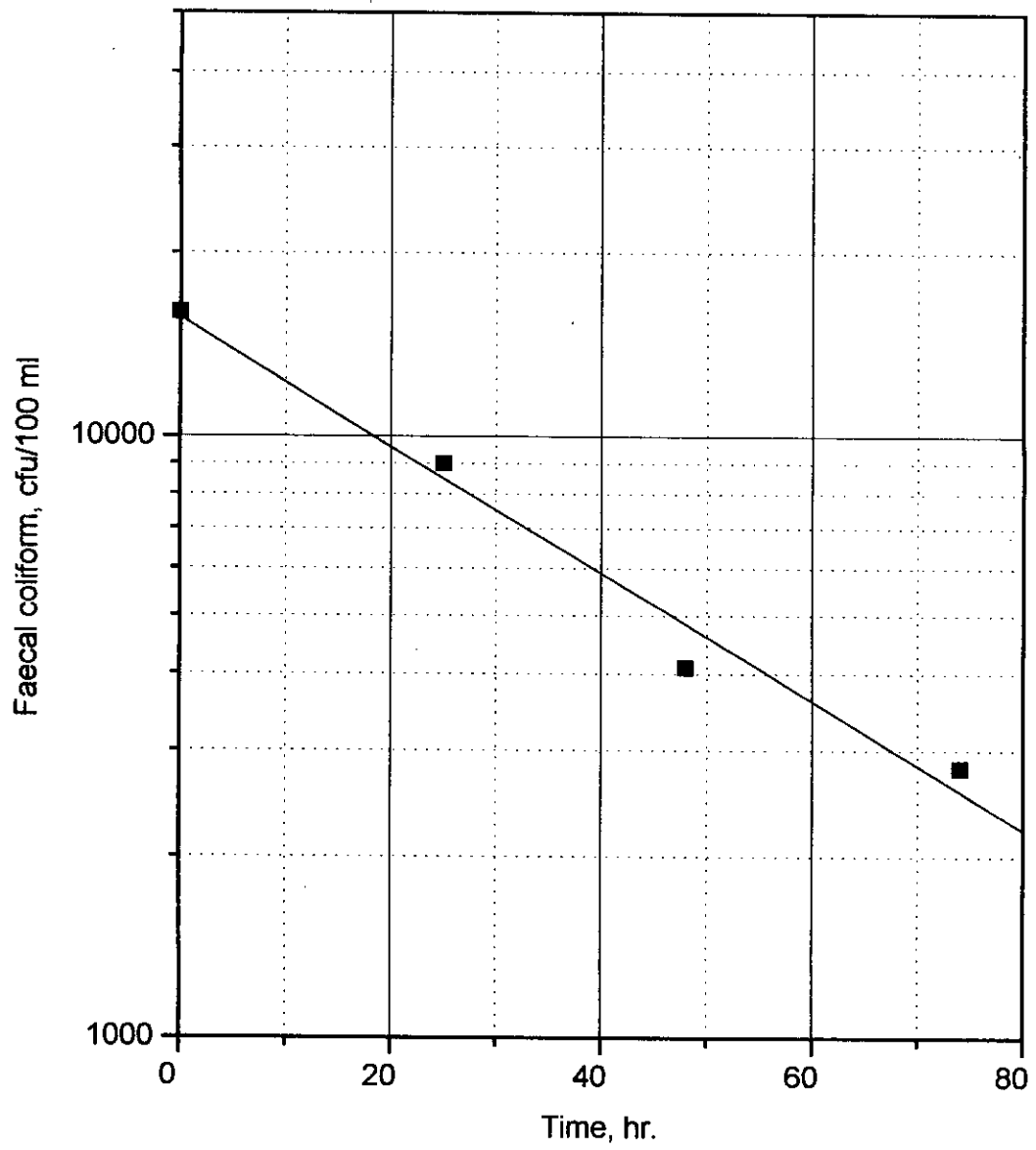
Fig. A.2 Decay of faecal coliform at 20^{0}C
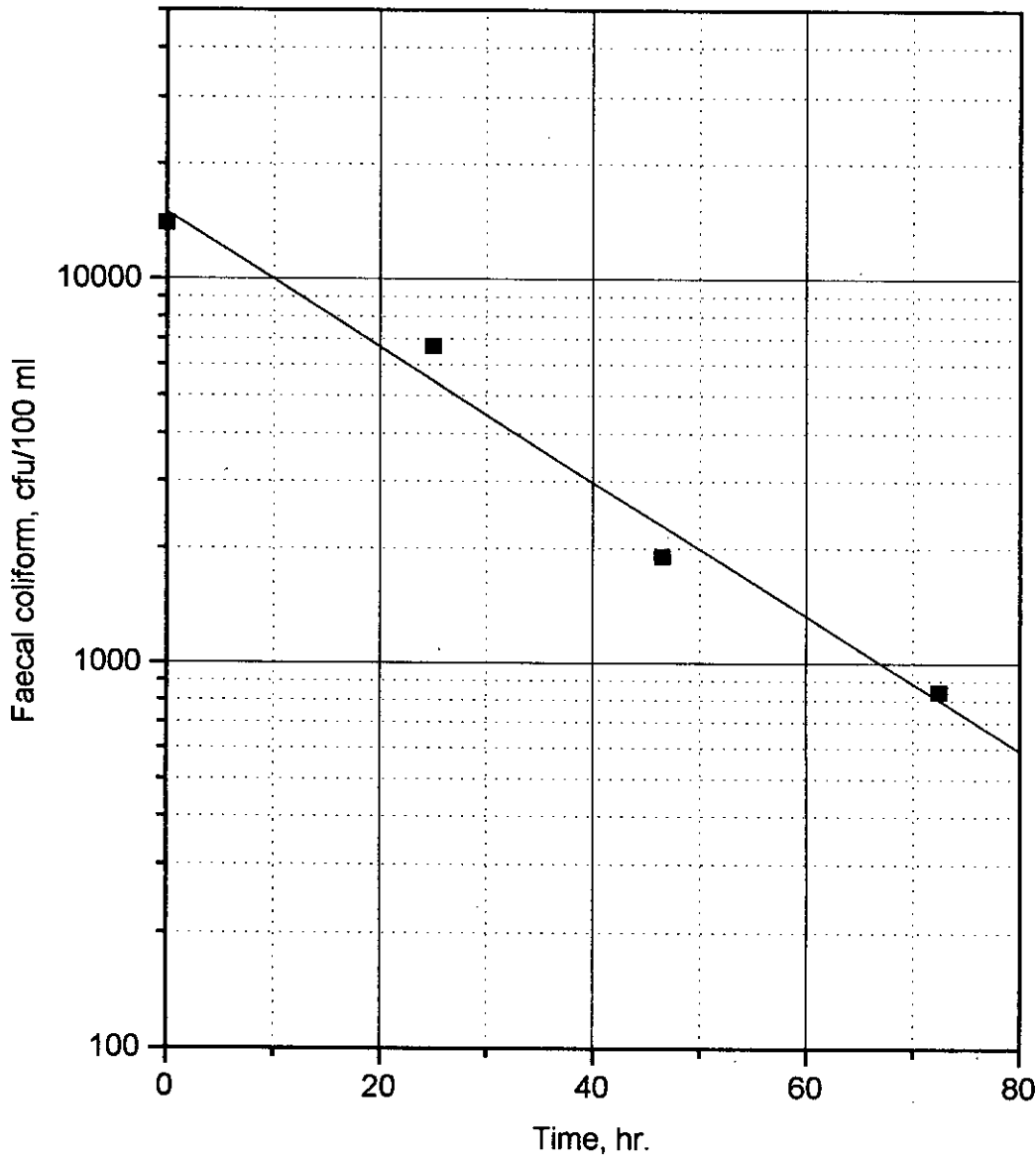
Fig. A.3 Decay of faecal coliform at 27^{0}C
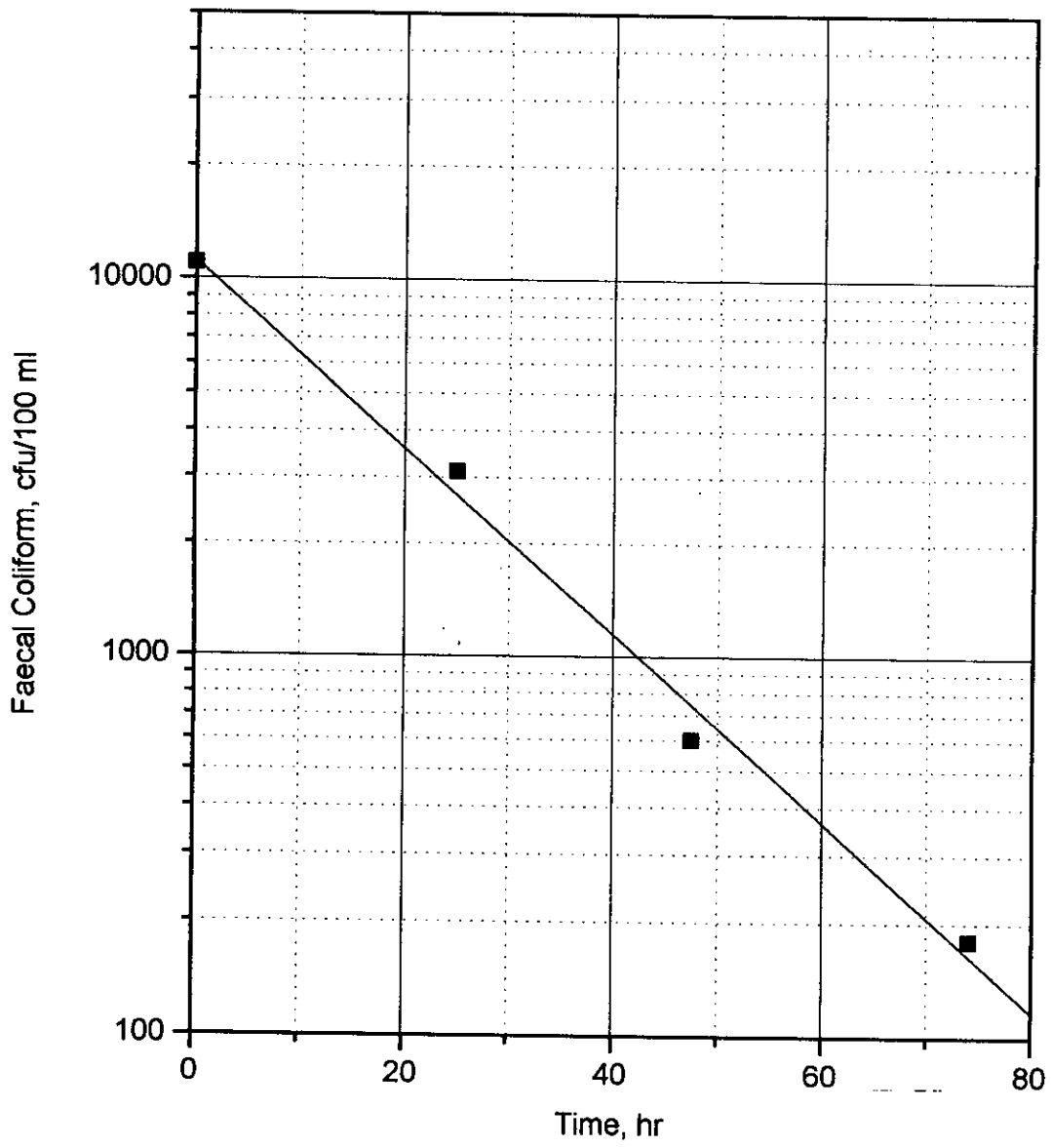
Fig. A-4 Decay of faecal coliform at 32⁰C
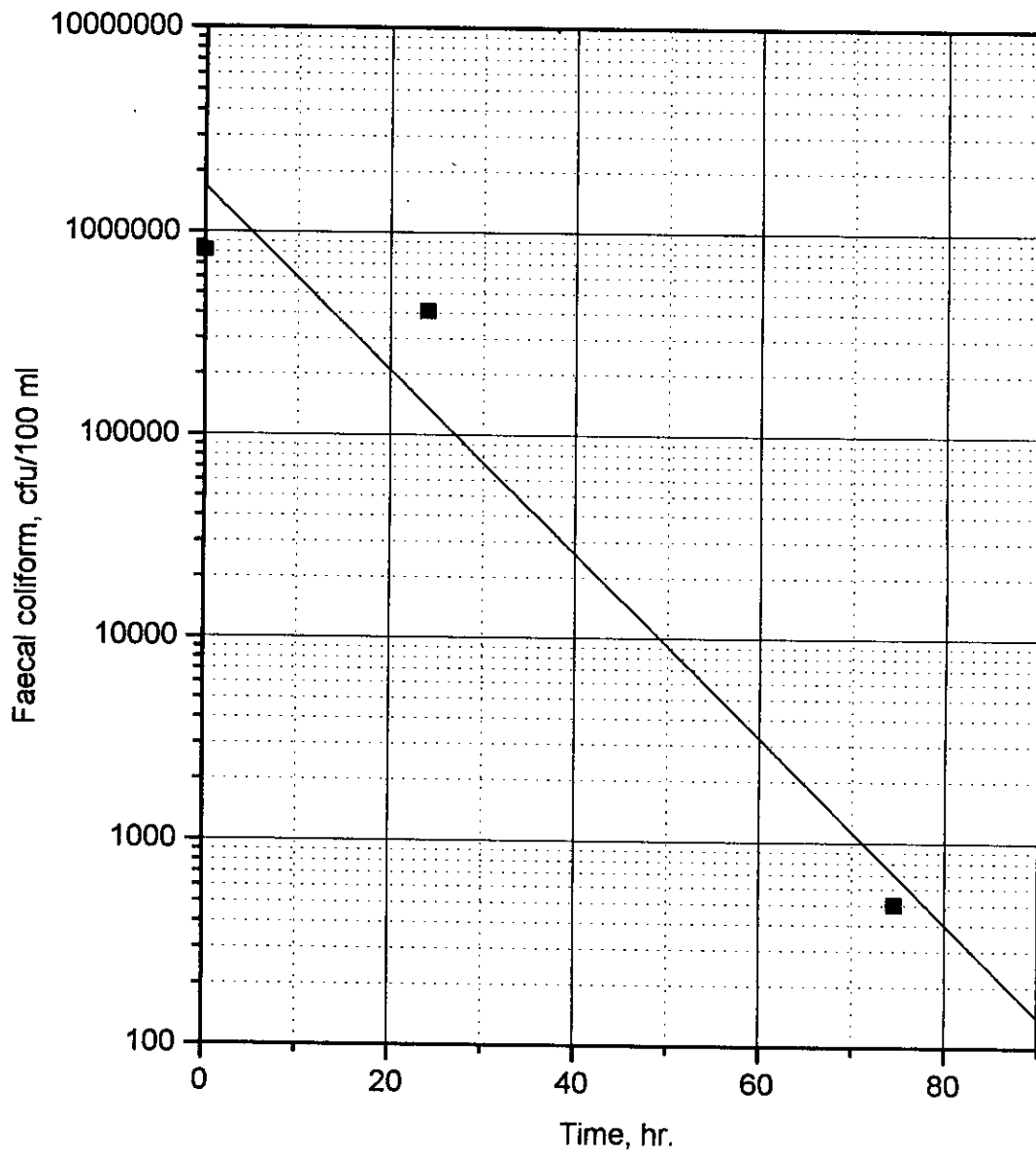
Fig. A.5 Decay of faecal coliform at pH 4.0
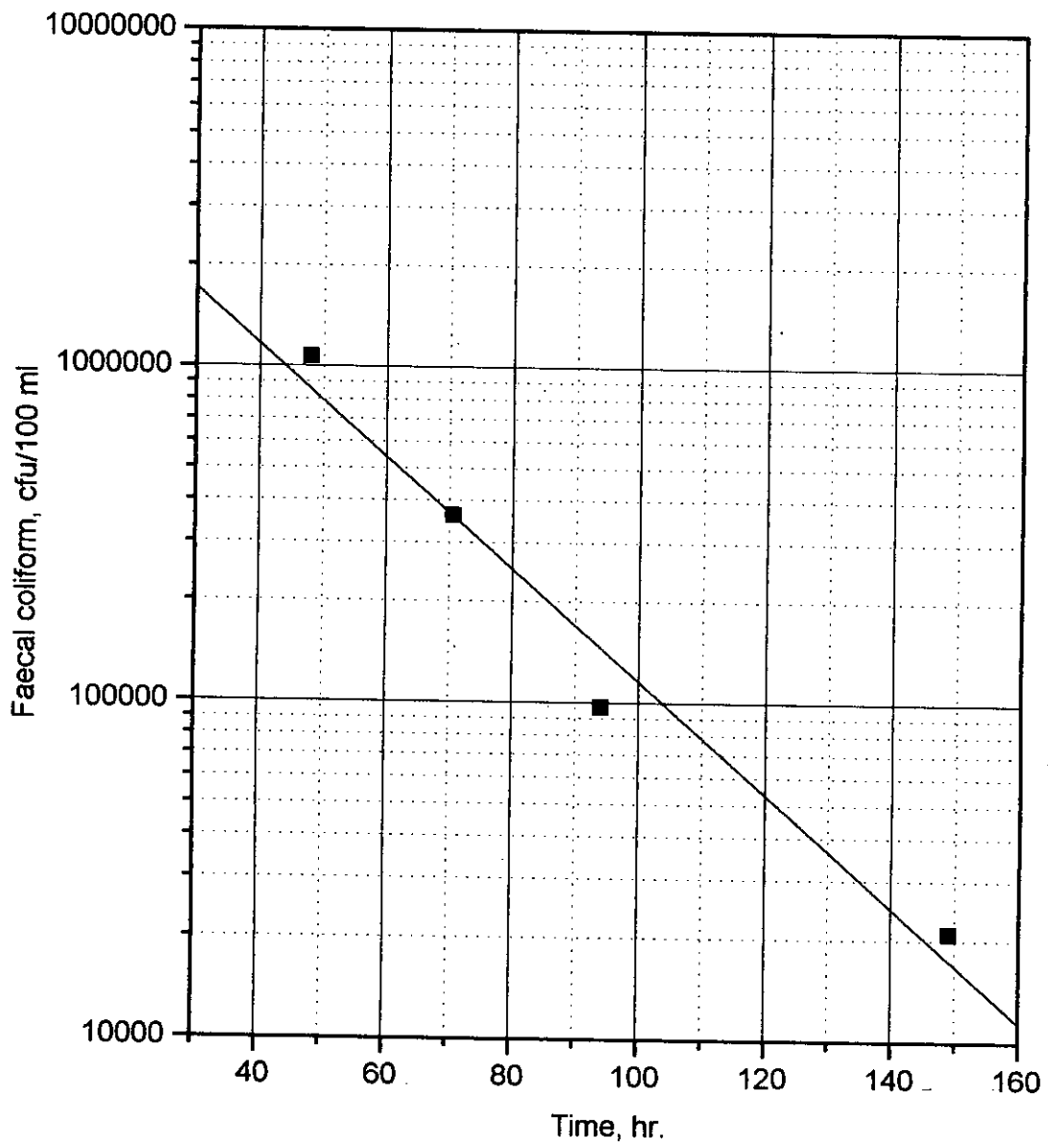
Fig. A.6 Decay of faecal Coliform at pH 6.0
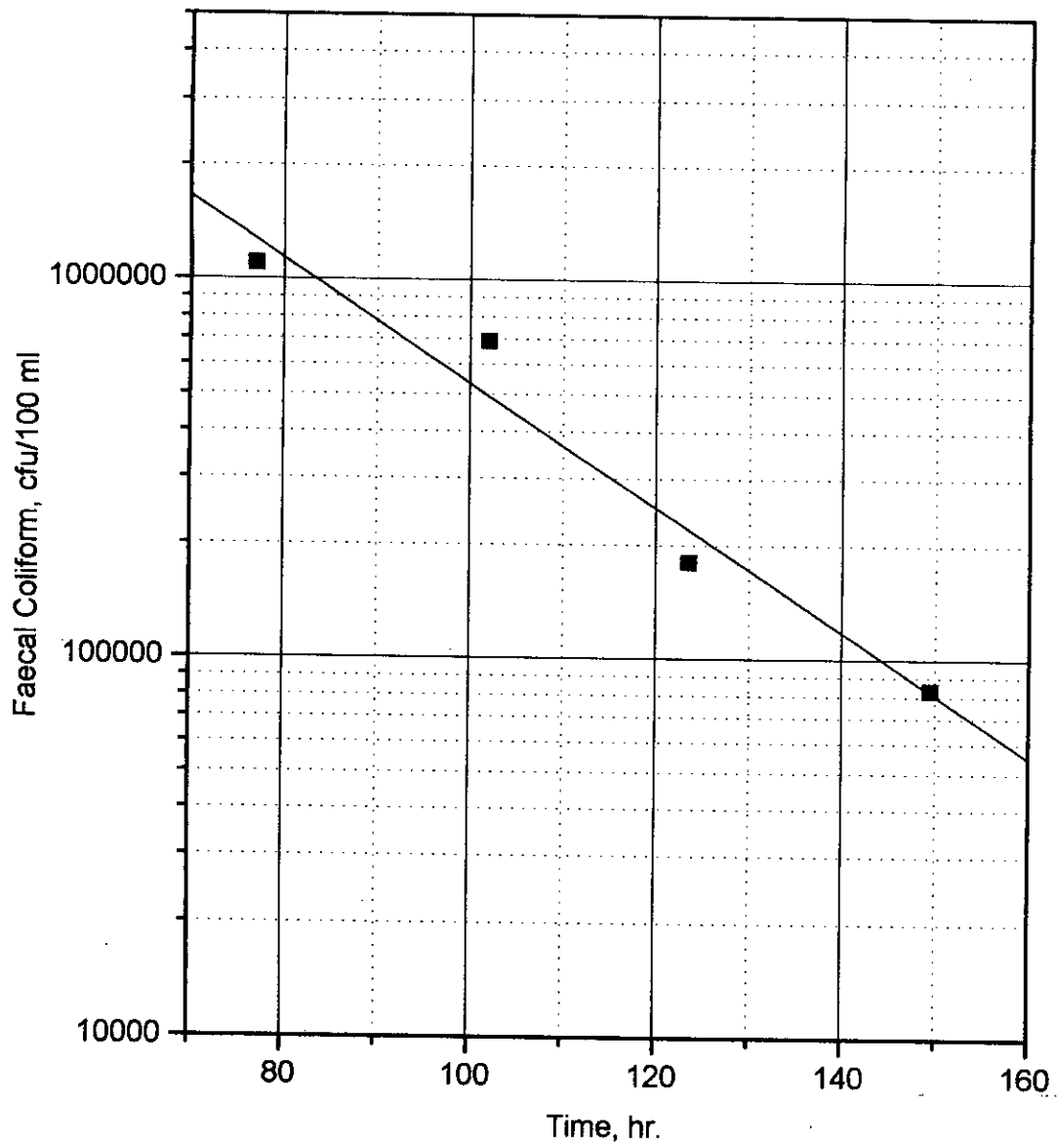
Fig. A.7 Decay of faecal coliform at pH 7.0
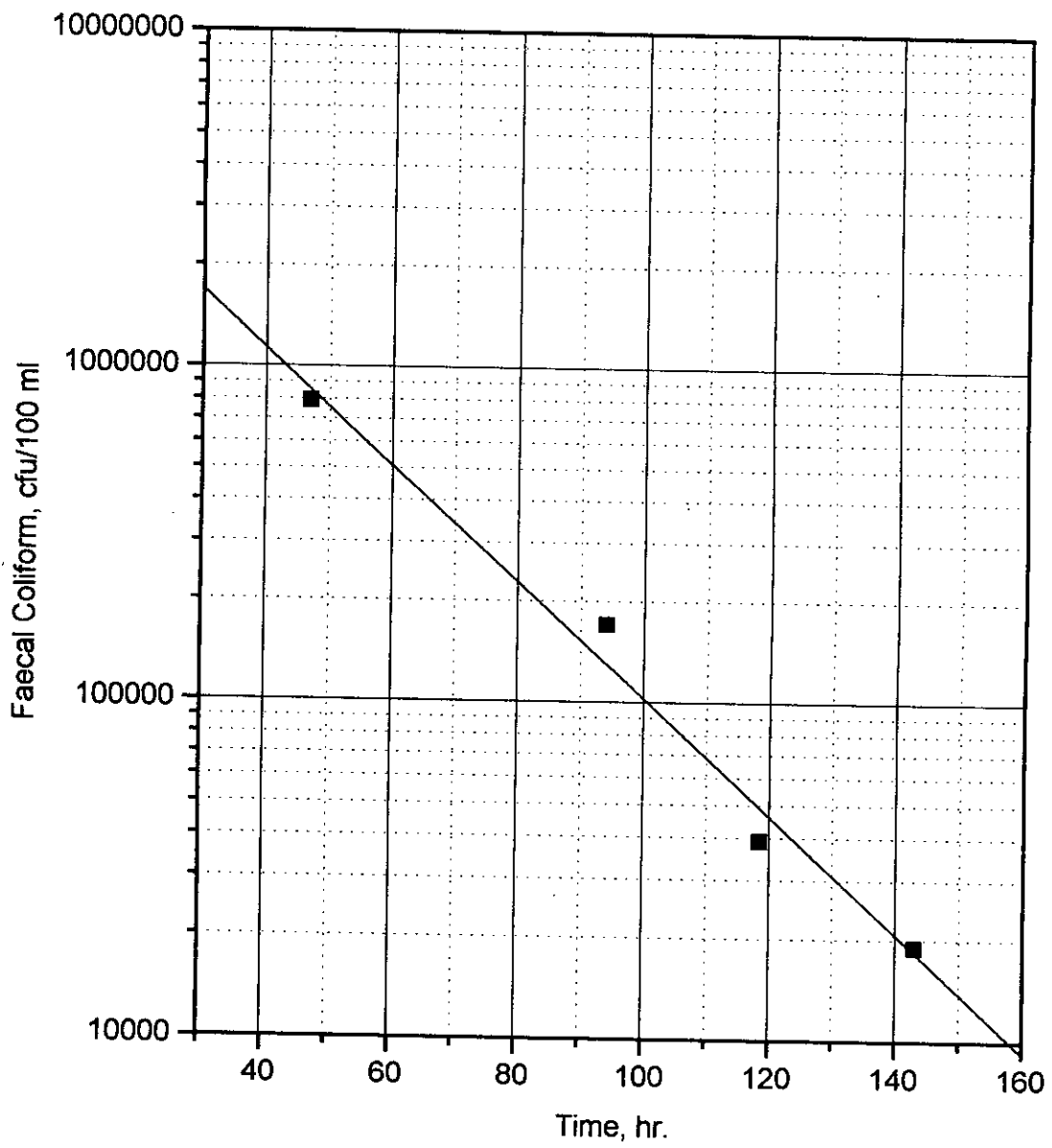
Fig. A.8 Decay of faecal coliform at pH 8.0
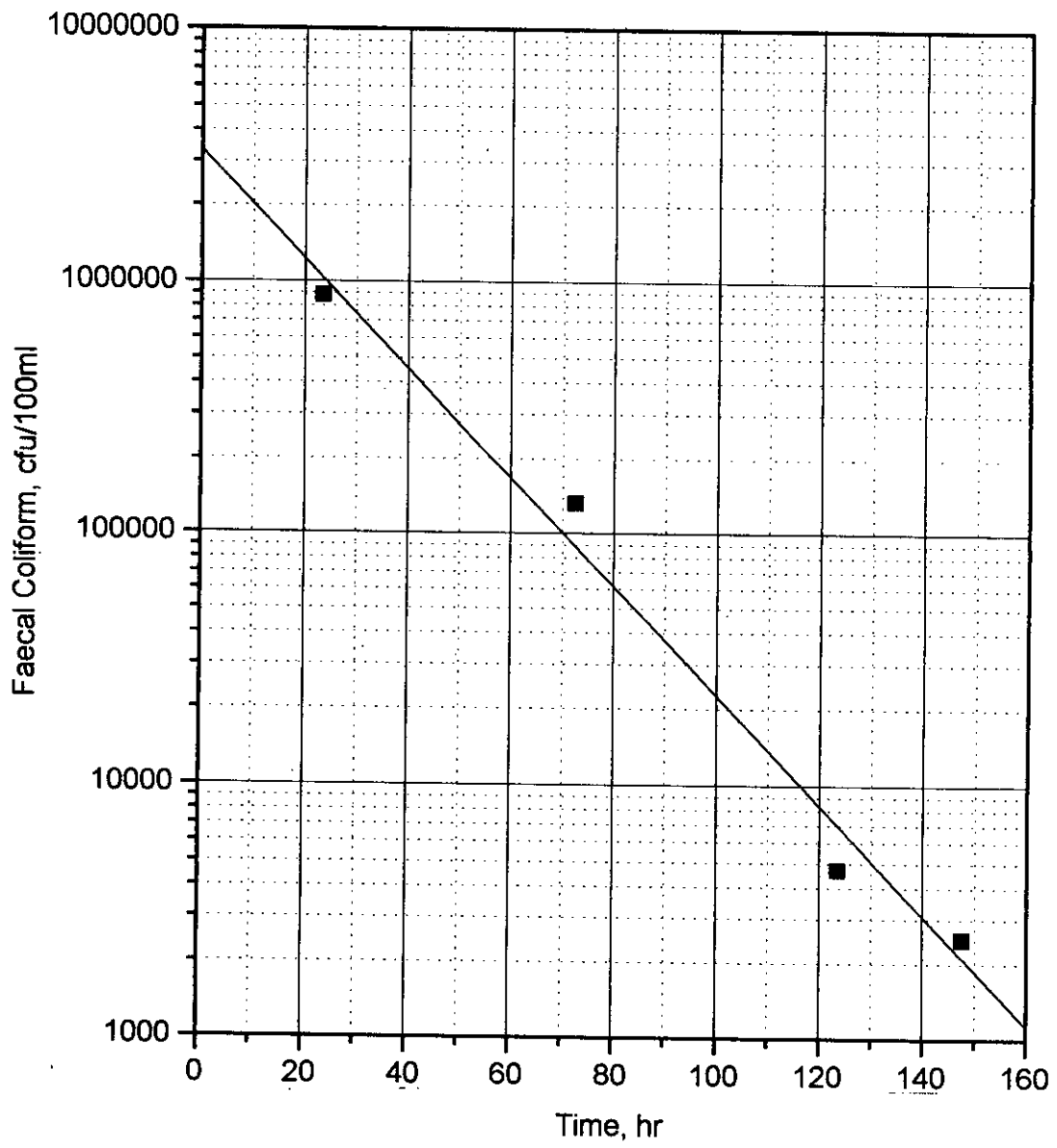
Fig. A.9 Decay of faecal coliform at pH 9.0

APPENDIX B

Flow Data at Diferent Chainage

Table B.1 Flow data at chainage 0.5 km

Time, Minute	Discharge, m^3/sec
0	0
14	27.57
44	79.31
74	111.4
104	152.94
134	181.44
164	216.05
194	261.44
224	288.77
254	293.29
284	257.69
314	206.55
344	141.67
374	33.04
404	-90.01
434	-205.3
464	-277.38
494	-281.17
524	-282.73
554	-316.28
584	-342.09
614	-333.02
644	-295.04
674	-256.37
704	-211.61
734	-131.62
764	-30.85
772	0

Table B.2 Flow data at chainage
4 km

Time, minute	Discharge, m^3/sec
0	0
21	28.4
51	73.46
81	106.91
111	145.53
141	163.55
171	198.21
201	249.95
231	243.06
261	218.33
291	188.07
321	131.8
351	67.91
381	-34.96
411	-146.85
441	-206.73
471	-230.17
501	-236.31
531	-270.21
561	-307.41
591	-291
621	-262.31
651	-236.53
681	-201.73
711	-154.59
741	-63.73
761	0

Table B.3 Flow data at chainage 6.5 km

Time, minute	Duscharge, m^3/sec
0	0
26	38.68
56	81.9
86	111.32
116	126.94
146	161.83
176	206.65
206	210.34
236	189.99
266	166.63
296	131
326	84.8
356	4
386	-96.52
416	-158.19
446	-186.43
476	-207.64
506	-241.64
536	-270.47
566	-263.04
596	-238.61
626	-217.23
656	-198.4
686	-161.25
716	-86.47
746	-3.11
747	0

Table B.4 Flow data at chainage
11 km

Time, Minute	Discharge, m^3/sec
0	0
10	15.24
40	65.69
70	87.49
100	103.79
130	139.53
160	175.33
190	188.68
220	171.92
250	150.94
280	131.52
310	94.15
340	28.35
370	-61.18
400	-126.33
430	-157.02
460	-189.71
490	-223.42
520	-244.32
550	-244.38
580	-223.06
610	-204.46
640	-196.1
670	-163.14
700	-101.38
730	-30.23
747	0

Table B.5 Flow data at chainage 14 km

Time, Minute	Discharge, m^3/sec
0	0
24	42.09
54	60.18
84	79.39
114	112.51
144	138.79
174	159.65
204	151.83
234	133.21
264	130.02
294	104
324	52.98
354	-17.48
384	-86.53
414	-125.16
444	-168.44
474	-202
504	-213.3
534	-220.37
564	-204.43
594	-192.21
624	-190.14
654	-162.37
684	-119.32
714	-59.65
743	0

Table B.6 Flow data at chainage
17 km

Time, Minute	Discharge, m^3/sec
0	0
3	3.83
33	31.47
63	54.94
93	74.86
123	101.19
153	118.98
183	124.88
213	121.07
243	119.81
273	116.62
303	80.57
333	29.1
363	-28.37
393	-95.77
423	-142.12
453	-170.31
483	-184.61
513	-182.71
543	-184.09
573	-183.79
603	-172.7
633	-164.48
663	-135.3
693	-91.33
723	-48.34
753	-3.13
755	0

APPENDIX C

Cross-sectional Profiles at diffeent sections

BURIGANGA
         23.500
COORDINATES
    1   90.358   23.721
FLOW DIRECTION
0
DATUM
       0.00
RADIUS TYPE
    0
PROFILE   43

0.00	7.08	1.00	<1>
0.00	6.90	1.00	
42.67	6.74	1.00	
45.70	4.44	1.00	
56.69	2.77	1.00	
62.79	4.44	1.00	
93.30	4.63	1.00	
160.30	5.03	1.00	
206.00	1.60	1.00	
213.66	1.40	1.00	
225.86	2.92	1.00	
234.70	1.39	1.00	
260.30	-1.66	1.00	
281.00	-3.18	1.00	
304.50	-4.70	1.00	
320.60	-6.23	1.00	
348.90	-7.75	1.00	<2>
381.30	-6.23	1.00	
415.58	-4.70	1.00	
452.00	-1.66	1.00	
480.67	1.69	1.00	
498.96	2.92	1.00	
514.80	2.30	1.00	
529.00	4.40	1.00	
534.90	4.80	1.00	
569.98	4.90	1.00	
575.50	5.20	1.00	
576.68	4.69	1.00	<3>
1335.20	2.99	20.00	
1780.26	3.05	20.00	
2225.33	3.42	20.00	
3115.46	3.51	20.00	
3560.52	4.06	20.00	
4005.59	4.09	20.00	
4450.65	4.12	20.00	
4895.72	4.33	20.00	
5340.78	4.42	20.00	
5785.85	4.73	20.00	
6230.91	4.82	20.00	
6675.98	4.85	20.00	
7121.05	5.03	20.00	
7566.11	5.67	20.00	
8011.18	8.48	20.00	

BURIGANGA
            27.000
COORDINATES
        1   90.401   23.708
FLOW DIRECTION
0
DATUM
        0.00
RADIUS TYPE
        0
PROFILE   37
        0.00   6.40   1.00   <1>
        0.00   6.13   1.00
       42.67   5.60   1.00
       91.40   5.54   1.00
       91.40   5.84   1.00
      132.89   4.68   1.00
      140.80   4.36   1.00
      152.40   1.31   1.00
      165.80   -1.74   1.00
      180.40   -3.26   1.00
      198.12   -4.79   1.00
      220.10   -6.31   1.00
      241.70   -6.30   1.00
      266.10   -7.80   1.00
      289.56   -7.80   1.00
      312.70   -9.36   1.00   <2>
      334.00   -6.31   1.00
      351.00   -3.26   1.00
      363.90   1.31   1.00
      373.00   4.36   1.00
      377.95   6.54   1.00   <3>
      429.19   2.59   20.00
      858.38   2.69   20.00
     1287.57   2.78   20.00
     1716.76   2.81   20.00
     2145.95   2.90   20.00
     2575.14   2.93   20.00
     3004.32   2.99   20.00
     3433.51   3.14   20.00
     3862.70   3.42   20.00
     4291.89   3.51   20.00
     4721.08   4.09   20.00
     5150.27   4.12   20.00
     5579.46   4.33   20.00
     6008.65   4.73   20.00
     6437.84   5.00   20.00
     6867.03   5.67   20.00

BURIGANGA
           29.500
COORDINATES
     1   90.417   23.692
FLOW DIRECTION
0
DATUM
        0.00
RADIUS TYPE
     0
PROFILE   36

0.00	5.18	1.00	<1>
0.00	5.06	1.00	
3.66	4.27	1.00	
21.34	1.92	1.00	
30.48	5.75	1.00	
58.22	5.29	1.00	
58.22	5.56	1.00	
119.20	4.47	1.00	
163.98	4.55	1.00	
168.25	4.27	1.00	
174.35	3.36	1.00	
183.20	2.14	1.00	
197.80	1.22	1.00	
207.60	-0.30	1.00	
217.30	0.62	1.00	
240.80	1.22	1.00	
264.87	-1.82	1.00	
296.30	-1.82	1.00	
333.45	-3.35	1.00	
376.43	-6.39	1.00	<2>
412.10	-4.87	1.00	
480.67	-3.35	1.00	
537.36	-1.82	1.00	
618.70	-2.75	1.00	
635.20	4.27	1.00	
640.08	6.41	1.00	
644.00	6.71	1.00	<3>
957.38	2.78	20.00	
1436.07	2.90	20.00	
1914.76	3.02	20.00	
2872.14	3.05	20.00	
3350.83	3.20	20.00	
3829.52	3.36	20.00	
4308.21	3.51	20.00	
4786.91	4.42	20.00	
5265.60	5.40	20.00	

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BURIGANGA
        34.000
COORDINATES
   1            90.450           23.671
FLOW DIRECTION
0
DATUM
      0.00
RADIUS TYPE
   0
PROFILE         36
     0.00        6.35       1.00        <1>
     0.00        6.09       1.00
     7.62        4.20       1.00
    23.47        2.07       1.00
    42.37        1.16       1.00
    62.79        1.77       1.00
    83.20        2.07       1.00
   106.07        1.77       1.00
   126.20        1.77       1.00
   147.20        1.16       1.00
   166.73        2.38       1.00
   181.96        4.20       1.00
   186.54        5.28       1.00
   247.50        5.53       1.00
   305.40        6.15       1.00
   309.06        4.11       1.00
   323.39        2.59       1.00
   335.89        1.07       1.00
   346.00       -0.46       1.00
   358.40       -0.46       1.00
   371.95       -1.98       1.00
   390.85       -3.50       1.00
   428.96       -6.55       1.00        <2>
   463.41       -5.03       1.00
   497.87       -3.51       1.00
   527.13       -1.98       1.00
   570.42       -0.46       1.00
   587.80       -1.07       1.00
   605.48        4.11       1.00
   619.20        5.67       1.00
   680.18        6.46       1.00
   728.65        5.94       1.00
   759.14        6.33       1.00
   771.64        5.32       1.00        <3>
  2967.69        3.02      20.00
  4451.53        3.36      20.00
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BURIGANGA
        37.000
COORDINATES
    1           90.467              23.650
FLOW DIRECTION
0
DATUM
       0.00
RADIUS TYPE
    0
PROFILE            38
       0.00        5.75        1.00        <1>
       6.10        7.08        1.00
      19.80        6.95        1.00
      53.34        5.15        1.00
      59.44        4.00        1.00
      71.02        2.48        1.00
      78.94        2.17        1.00
      90.53        0.95        1.00
     118.26       -1.48        1.00
     137.16       -3.92        1.00
     144.78       -5.14        1.00
     150.57       -4.84        1.00
     175.56       -5.14        1.00
     234.70       -5.14        1.00        <2>
     292.90       -0.57        1.00
     356.00        0.95        1.00
     370.00        4.00        1.00
     377.95        6.44        1.00
     408.40        6.72        1.00
     438.90        6.70        1.00
     443.48        3.99        1.00
     466.30        1.86        1.00
     493.47        0.03        1.00
     551.00       -0.43        1.00
     609.30       -0.43        1.00
     671.17        0.03        1.00
     728.47        0.34        1.00
     781.80        1.55        1.00
     832.70        3.38        1.00
     877.50        3.38        1.00
     910.49        3.69        1.00
     932.69        3.99        1.00        <3>
    1002.79        3.81        1.00
    1082.04        3.68        1.00
    1112.50        3.66        1.00
    1139.93        3.72        1.00
    1139.93        3.66        1.00
    2452.16        3.50       20.00
```

```
BURIGANGA
        40.000
COORDINATES
    1           90.458          23.633
FLOW DIRECTION
0
DATUM
        0.00
RADIUS TYPE
    0
PROFILE              58
        0.00        4.32        1.00        <1>
        0.00        4.21        1.00
       60.96        3.93        1.00
      121.92        3.93        1.00
      182.88        3.97        1.00
      224.00        3.96        1.00
      224.00        4.04        1.00
      284.98        4.03        1.00
      315.47        3.93        1.00
      344.40        3.93        1.00
      381.61        4.04        1.00
      382.50        3.90        1.00
      396.20        2.68        1.00
      411.48        2.68        1.00
      426.72        2.99        1.00
      448.36        2.07        1.00
      453.24        2.68        1.00
      457.20        2.99        1.00
      472.40        2.68        1.00
      487.68        2.66        1.00
      502.90        2.99        1.00
      517.86        3.80        1.00
      519.38        4.24        1.00
      521.50        3.89        1.00
      546.50        1.75        1.00
      593.14        0.84        1.00
      632.46        0.84        1.00
      670.86        0.53        1.00
      727.87        0.84        1.00
      777.24        0.84        1.00
      848.87       -0.38        1.00
      925.37       -0.69        1.00
     1010.40        1.75        1.00
     1073.80        0.84        1.00
     1127.15        0.84        1.00
     1207.30        2.67        1.00
     1239.60        2.06        1.00
     1258.80        2.67        1.00
     1273.76        2.67        1.00
     1305.15        1.75        1.00
     1325.88        0.23        1.00
     1380.13       -0.69        1.00
     1439.29       -3.73        1.00
     1484.00       -5.87        1.00
     1523.69       -5.87        1.00        <2>
     1568.50       -5.26        1.00
     1585.87       -4.65        1.00
     1613.60       -3.73        1.00
     1630.98       -3.73        1.00
     1651.70       -2.21        1.00
     1662.38        3.89        1.00
     1676.40        3.89        1.00
```

```
   1696.20        5.36        1.00
   1748.00        4.75        1.00
   1808.98        5.54        1.00
   1814.47        4.63        1.00        <3>
   3289.68        4.39       20.00
   4934.51        4.88       20.00
*******************************
```
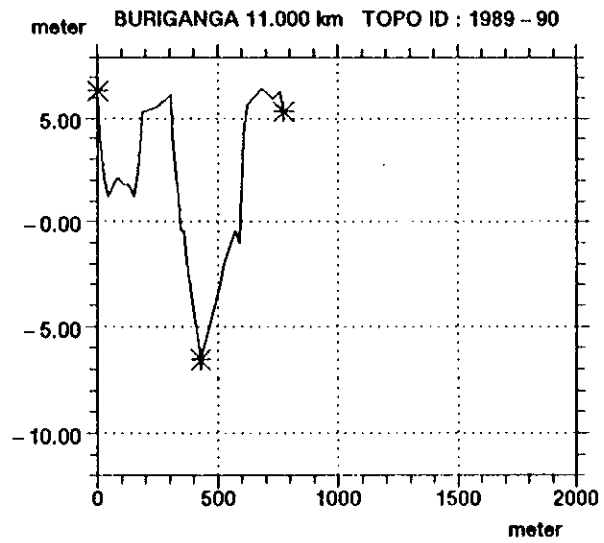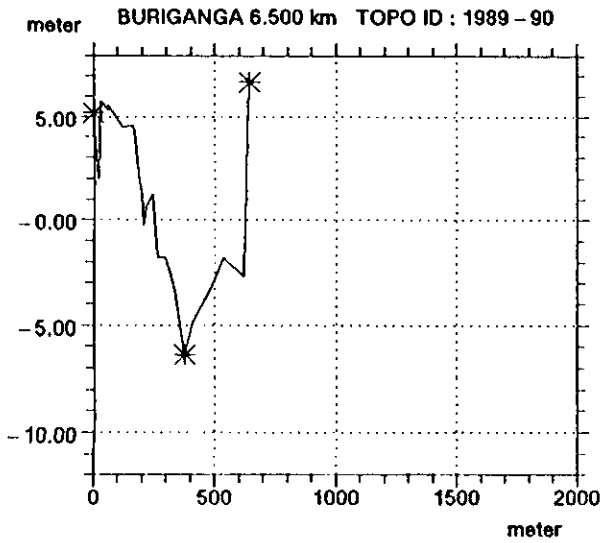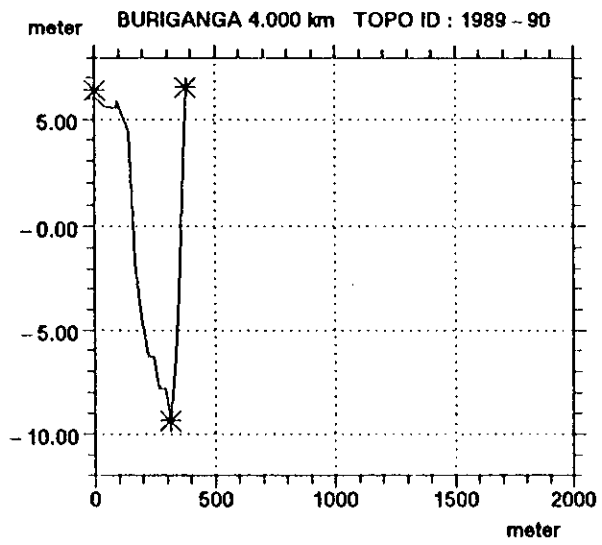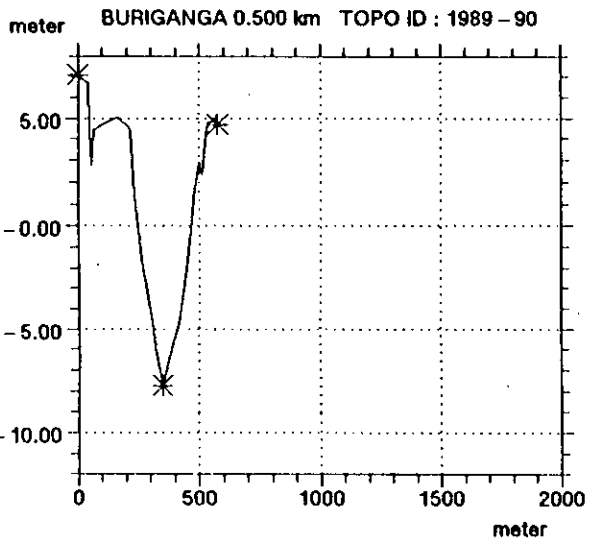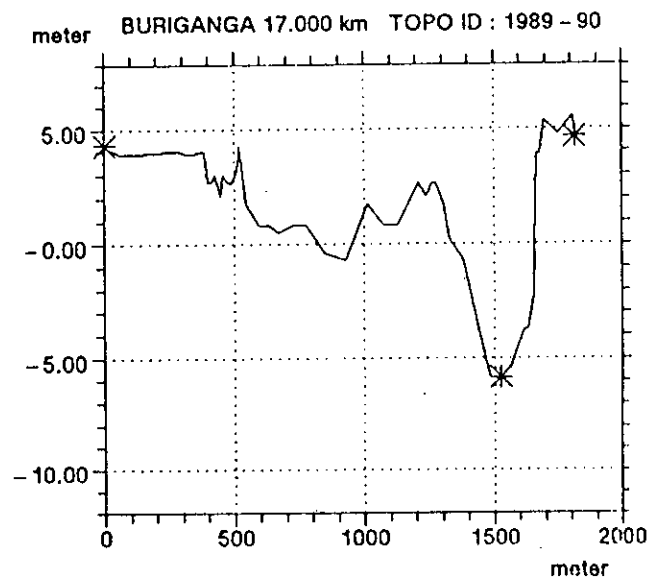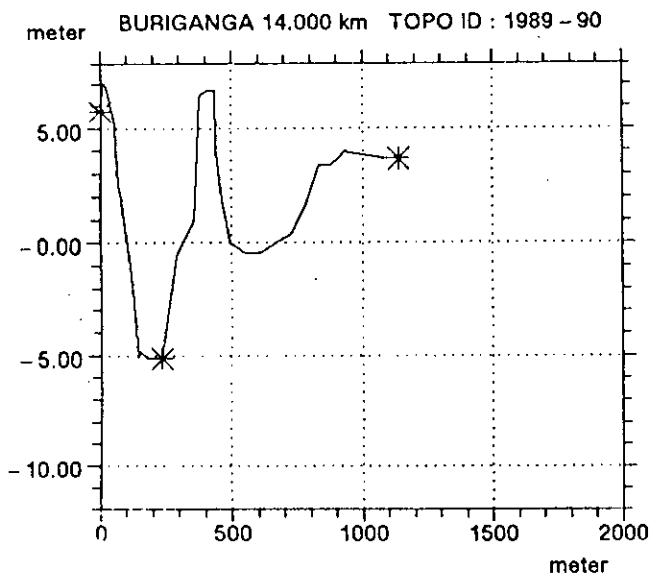
Figure 5.12: Cross sections of the Buriganga at chainages
0.5, 4, 6.5 and 11 kms.

DATA BASE : TEM

MIKE 11

Dwg no.:

C-8

Figure 5.13: Cross sections of the Buriganga at chainages
14 and 17 kms.

	MIKE 11
	Dwg no.:
DATA BASE : TEM	

C-9