Microbial evaluation of Al-Khobar wastewater treatment plants.

Muhammad Saleem

Civil Engineering

1997

Abstract

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Microbial Evaluation of Al-Khobar Wastewater Treatment Plant

by

Muhammad Saleem

A Thesis Presented to the

FACULTY OF THE COLLEGE OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
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MASTER OF SCIENCE

In

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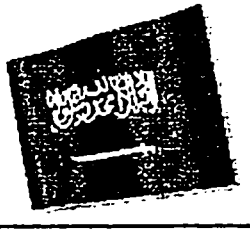

بسم الله الرحمن الرحيم

King Fahd University of Petroleum & Minerals

جامعة الملك فهد للبترول والمعادن

Dedicated to

MY BELOVED PARENTS

ACKNOWLEDGMENTS

All praise be to Allah Subhanahu wa Ta'ala, the Lord of the Worlds, for having guided me at every stage of my life.

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TABLE OF CONTENTS

LIST OF FIGURE

LIST OF TABLES

THESIS ABSTRACT

NAME OF STUDENT	Muhammad Saleem
TITLE OF STUDY	Microbial Evaluation of Al-Khobar Wastewater Treatment Plant.
MAJOR FIELD	Civil Engineering
DATE OF DEGREE	December, 1997

A one year study was conducted at the Al-Khobar Wastewater Treatment Plant to investigate the seasonal variations of temperature and flowrate on the fate of indicator microorganisms. The raw sewage, secondary effluent, chlorinated effluent, and dry sludge were analyzed for the detection and enumeration of five indicator microorganisms, namely; Standard Plate Count, total coliform, fecal coliform, coliphage, and *Clostridium perfringens* on a weekly basis. Multiple non-linear stepwise regression analysis was performed to correlate the microbial population with temperature and flowrate of wastewater, measured in the treatment plant to develop regressional models for all studied microorganisms at different stages. Verification of these models was performed on the basis of percent error and plot of measured Vs predicted values.

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Master Of Science Degree

King Fahd University Of Petroleum & Minerals

Dhahran, Saudi Arabia

December, 1997

خلاصة الرسالة

اسم الطالب: محمد سليم

عنوان الرسالة: تقييم الميكروبات في محطة تنقية مياه الصرف الصحي بالخبر

التخصص: هندسة مدنية (هندسة مصادر المياه والبيئة)

تاريخ الدرجة: ديسمبر ١٩٩٧م

لقد تمت في محطة تنقية مياه الصرف الصحي بالخبر دراسة استغرقت سنة وكان غرضها دراسة تأثير التغيرات الموسمية (درجة الحرارة، والتدفق) على مصير الكائنات الحية الدقيقة المؤشرة. وقد تم كشف وإحصاء خمسة كائنات حية (العد الصحني ، والكوليفورم الكلي، والكوليفورم الغائطي، والكلوليفاج، والكولوستيريديوم بيرفيرينجس) في مياه الصرف الصحي غير المعالجة، والمياه المعالجة ثانوياً، والمياه المعالجة بالكلور، والحمأة الجافة وذلك بصورة أسبوعية.

لقد جرى تحليل بواسطة الإنحدار الخطي لربط الميكروبات بدرجة الحرارة وتدفق مياه الصرف الصحي المقاس في محطة التنقية بهدف تطوير نموذج رياضي يستطيع توقع تراكيز الكائنات الحية التي درست وعلى مختلف المراحل. وتم التحقق من هذا النموذج على أساس حساب الخطأ المئوي ورسم القيم المقاسة مع تلك المتوقعة.

إن نتيجة الاختبار (t-test) الإحصائي الذي أقيم على متوسط كثافة الكائنات الحية المذكورة أظهرت أعلى كثافة في الصيف بالمقارنة بالشتاء، أما بالنسبة للحمأة الجافة فقد أظهرت النتائج كثافة عالية في الشتاء لبعض الكائنات (الكوليفورم الكلي، الكوليفورم الغائطي، والكوليفاج) بينما كثافة العد الصحني، والكلوستيريديزم بيرفيرينجس لم يظهر عليها تأثر نتيجة التغيرات الموسمية. كما لوحظ وجود نسبة مئوية عالية في إزالة العد الصحني والكوليفورم الكلي والكوليفورم الغائطي (من ٩٩% إلى ٩٩,٩%) في المياه المعالجة ثانوياً بالمقارنة مع الكوليفاج (٨٣,٦%) والكلوستيريديوم بيرفيرينجس (٥٥,٥%)، أما بالنسبة للعد الصحني والكوليفورم الكلي والكوليفورم الغائطي فإن نسبة الإزالة كانت ٩٩,٧%، أما الكوليفاج، والكلوستيريديوم فقد أزيلت بنسبة ٥٢%، و ٤٢% على التوالي. الجدير بالذكر أن الكوليفاج والكلوستيريديوم أظهرا مقاومة عالية ضد الكلور.

درجة الماجستير

جامعة الملك فهد للبترول والمعادن

الظهران - المملكة العربية السعودية

ديسمبر ١٩٩٧م

CHAPTER 1

1. INTRODUCTION

Wastewater generated, if not properly treated, can cause pollution when it is ultimately disposed off on land or in receiving waters. Presence of pathogenic microorganisms in the wastewater effluents pose a danger to the living environment. In addition, the reuse of wastewater is becoming an important and economical option for conserving the water resources and decreasing pollution in the receiving waters. The ultimate goal of wastewater treatment and management is the protection of the environment keeping in view the economical, social, and political concerns (Geldreich et al., 1968).

Studies indicate that a large number of pathogenic microorganisms are found in wastewaters (Feachem et al., 1983 and Mara 1974). There are several approaches for determining the possible presence of pathogens. The most common approach is the use of indicator microorganisms; organisms whose presence suggests that pathogens may also be present. The presence of these pathogens can be confirmed by the use of indicator microorganisms

such as coliform, fecal coliform etc. Population densities of these indicator microorganisms represent the extent of pathogenity of the wastewater.

According to Kawamura and Kaneko (1986), changes in microbial population densities, and their distribution patterns, reflect the purification effects in the course of treatment performance. The microbial population density is directly related to the efficiency of biological treatment of the wastewater. It defines the treatment efficiency and plant performance.

Population density of microorganisms in wastewater treatment systems vary seasonally. Rao *et al.*, (1974), during a two year study on virus removal in an activated sludge sewage treatment, located in Bombay, reported a higher population density of viruses during summer. Once the change in removal efficiencies due to seasonal variations is determined, a fine tuning in the treatment system could be achieved by adjusting the operational parameters. For example, if less removal of coliform is achieved in secondary clarifier during winter, removal efficiency can be improved by providing relatively more detention time. On the basis of these studies guidelines may be developed which apply to other similar treatment plants in the region.

Generally, coliform and fecal coliform bacteria are used as the criterion to judge the public health safety from fecal pollution (MAW, 1989). However, many researchers (Kanemasa et al., 1986; Kawamura et al., 1985; Ashidate et al., 1985; Hirata et al., 1991) suggest that it is insufficient to judge the safety of water on the basis of their coliform densities. The existence of pathogenic

viruses able to survive sewage treatment processes have also been reported (IAWP&C study Group, 1983; Omura et al. 1987). Therefore, the evaluation for a more reliable indicator microorganism is highly recommended.

A one year study at Al-Khobar wastewater treatment plant was conducted to investigate seasonal variations of temperature and flow rate on the fate of five selected indicator microorganisms. Generally the climatic condition in the eastern province of Saudi Arabia can be broadly divided in two seasons, summer and winter. The summer season lasts from April to October and winter from November to March (Farooq et al., 1997). Detailed microbial analysis of different effluents from the Al-Khobar wastewater treatment plant have been made on weekly basis. Five different indicator microorganisms; namely, Standard Plate Counts, total coliforms, fecal coliforms, *Clostridium perfringens*, and coliphages have been used in this research to study their fate within the carrousel type activated sludge treatment plant. The microbial population of indicator microorganisms have been used to evaluate the treatment efficiency of Al-Khobar wastewater treatment plant during summer and winter.

Generally, the sludge drying beds are the final destination in treatment by the activated sludge. Dry sludge from activated sludge treatment plants is being used as a fertilizer in different cities of the Kingdom. Pathogens present in dry sludge may remain active even after a drying cycle of two weeks (Wellings et al., 1976). These circumstances increase the probability of direct contact between dry sludge and humans.

Previous studies carried out at Al-Khobar sewage treatment plant investigated the removal of microorganisms in the final effluents. The present study included the evaluation of the fate of indicator microorganisms in the dry sludge. The effect of seasonal variations of Saudi Arabian climate on the microbial population has also been studied. The insight gained in the treatment performance of Al-Khobar wastewater treatment plant may be applied to other activated sludge treatment plants operating in this region.

CHAPTER 2

2. LITERATURE REVIEW

2. 1 Wastewater Generation in the Kingdom of Saudi Arabia

In recent years, the amount of wastewater generated in the Kingdom of Saudi Arabia has reached 1000 $M.m^3$ / year. The volume of wastewater generated is expected to grow to 1500 $M.m^3$ / year by the year 2000 AD (Abu Rizaiza et al., 1994). This wastewater results from domestic, industrial, and commercial activities. The wastewater generated is treated in various treatment plants located in different cities of Kingdom. A summary of some major wastewater treatment plants of the Saudi Arabia is presented in Table 2.1. Only 150 $M.m^3$ / year of wastewater is reused, the rest being discharged in the desert or in the Arabian Gulf. Water and Sewage Authority is now coordinating and planning for a total use of the recycled wastewater and solid wastes as one of its main future objective (Al-Elaiw, 1994).

Table 2.1: Wastewater Treatment Plants in the Kingdom of Saudi Arabia

City	Treatment Plant	Plant Capacity (m^3/day)		Type of Treatment
		Design	Actual	
Al-Khobar	Al-Khobar STP	133,330	100,000	Carrousel activated sludge
Dammam	Dammam STP	180,000	160,000	Carrousel activated sludge
Al-Qatif	Al-Qatif STP	225,000	50,000	Carrousel activated sludge
Jeddah	Plant A	32,000	26,300	Activated sludge
Jeddah	Plant C	40,000	50,400	Activated sludge
Jeddah	Al-Komra	43,000	36,000	Trickling filters
Jeddah	Bani Malik	8,000	1,400	Activated sludge
Jeddah	Al-Jamia	8,000	3,400	Activated sludge
Makkah	Makkah TP	74,000	30,000	Trickling filters & activated sludge
Taif	Taif TP	67,000	146,400	Extended aeration
Madinah	Madinah TP	120,000	40,000	Activated sludge
Khamis Mushait	Khamis Mushait TP	9,000	5,616	Extended aeration
Abha	Abha TP	9,000	2,838	Extended aeration
Riyadh	Al-Hayer	200,000	358,000	Trickling filters
Unaiza	Unaiza TP	7,080	9,900	Extended aeration

After Abu Rizaiza et al., 1994

2. 1 .1 Wastewater Generation in the Eastern Province of the Kingdom of Saudi Arabia

The eastern province of Saudi Arabia has a land area of 1,000,000 Km^2, with an urban population of about 2.27 million which is expected to reach 4.0 million by the year 2000 AD. The treated wastewater quantity has been estimated at 512,000 m^3/day of which only 7.1% is being reused. Major wastewater treatment plants of eastern province are located in Dammam, Al-Khobar, Qatif, Jubail, Abqaiq, and Dhahran North. Typically all the newly designed secondary wastewater treatment plants are capable of giving good quality of effluent. About 22,000 m^3/day of treated wastewater is being used for irrigation inside the wastewater treatment plants (Al-Elaiw, 1994).

2. 1 .2 The Al-Khobar Wastewater Treatment Plant

Al- Khobar wastewater treatment plant is designed as a *carrousel* system and it can handle a daily flow of 133,300 m^3/d (35.25 MGD). The principal components of the plant are an inlet structure with screening, grit removal and flow measurement facilities, carrousel type aeration tanks, final clarifiers, sludge recirculation pumping stations, effluent storage lagoons, chlorinating facility, sludge thickeners, thickened sludge pumping station and sludge drying beds.

The wastewater treatment plant has the capability to handle an organic loading of 19,600 kg BOD per day and a suspended solids loading of about 26,700 kg/day at a peak flow of 240,000 m^3/d. Detention time at average and

peak flow used for designing was 17 and 9.4 hours respectively. Typical operating temperatures have been recorded in the range of 15°C to 35°C.

Another important parameter of this plant is the F/M ratio, which has been reported as 0.05 kg BOD/kg MLSS. The wastewater treatment plant is designed as an extended aeration system, the oxygen requirement is about 60,123 kg/day and for this purpose 18 aerators have been installed in six aeration tanks. In this system primary clarifiers are not present and six secondary clarifiers have been provided to handle average and peak daily flow of 133,330 and 233,330 m^3 /d respectively. The average detention time in the clarifiers is about 3.7 hours to get a clear effluent and a good removal efficiency.

The influent flows into the inlet works, which is divided into four channels in which mechanical screens are installed. After screening the sewage passes through four parallel partial flumes and the influent is led to the grit collection chambers. The wastewater overflows from V-notch type effluent weirs and is led to the aeration chambers for further treatment. In the aeration tank it combines with a constantly mixed mixture of activated sludge and wastewater.

In the final clarifiers the reduced velocity of flow induces the settlement of sludge in the bottom of the tank. The clear effluent overflows the weir plates around the periphery into the effluent channel. Finally the effluent after chlorination is discharged into the Arabian Gulf. The sludge that settle in the central hopper of the final clarifiers is called activated sludge which is recycled to the aeration tanks. The sludge so recycled is called the returned activated

sludge. Only an adequate amount of sludge is returned to the reactors and the rest is wasted. The thickened sludge is drawn off the central hopper by the sludge pumps to the sludge drying beds for drying. Lime is added to increase the pH of the sludge to achieve enhanced inactivation of microorganisms. As a final treatment sludge remain over the drying beds for more than 14 days, (Farooq & Nakhla, 1996).

2. 2 Importance of Wastewater Treatment

Climatic or seasonal changes determine the amount of water consumed which in turn determines the quantity and quality of the wastewater produced. Wastewater flow rates may also change due to the existence of seasonal recreational sites in an area (MetCalf and Eddy, 1991). Generally, in hot climate areas like Saudi Arabia flow rate of wastewater increases during summer, as the water use is at its peak during this period (MetCalf and Eddy, 1991). The water consumption in Saudi Arabia ranges from 300 to 350 liters per capita per day (Al-Elaiw, 1994).

Seasonal variation of temperature and flow in a wastewater treatment plant has a significant role on its treatment efficiency. Concentration of pathogens in sewage may vary seasonally. The incidence of infection in the population, may cause sudden or drastic changes in the pathogens reaching the wastewater treatment plant, (Gaudy & Gaudy, 1980).

Sewage contains very large numbers of organisms some of which are pathogenic to men and must be removed or at least reduced as far as possible to protect the human environment and prevent a cycle of infection, (Gerald and MetCalf, 1978).

The importance of wastewater treatment is related to the health of the community. Many studies on the ecology of wastewater treatment processes have been done in the past, Hawkes, (1963) reported that "although properly operated wastewater plants employing chlorination as a final disinfection stage can reduce pathogens concentrations by many orders of magnitude, however, it is still virtually impossible to assume complete elimination of pathogens".

Properly treated municipal wastewater is one of the best source of water for greening and landscaping purposes because the salinity is relatively low and the water contains both organic and inorganic materials with nutrient value.

2. 2 .1 Types of Wastewater Treatment

Methods of treatment in which the application of physical forces predominates are known as unit operations. Methods of treatment in which the removal of contaminants is bought about by chemical or biological reactions are known as unit processes. At the present time, unit operations and processes are grouped together to provide what is known as primary, secondary, and advanced (or tertiary) treatment. In primary treatment, physical operations such as screening and sedimentation are used to remove the floating and settleable

solids found in wastewater. In secondary treatment, biological and chemical processes are used to remove most of the organic matter. In advanced treatment, additional combinations of unit operations and processes are used to remove other constituents, such as nitrogen and phosphorous, that are not reduced significantly by secondary treatment.

2. 2 .2 Biological Treatment of Wastewater

Treatment methods in which the removal of contaminants is bought about by biological activity are known as biological unit processes. Biological treatment is used primarily to remove the biodegradable organic substances (colloidal or dissolved) in wastewater. Basically, these substances are converted into gases that can released to the atmosphere and into biological cell tissue that can be removed by settling. Biological treatment is also used to remove nutrients in wastewater. With proper environmental control, wastewater can be treated biologically in most cases.

2. 2 .3 Activated Sludge Treatment Process

The activated sludge process is among the most widely used biological processes for the treatment of domestic and industrial wastewaters. A number of variations of the basic system have been developed over the years which give the process a versatility which enables it to be adopted to a wide range of operational circumstances, (Winkler, 1981).

2. 2 .3 .1 Basic Operation Principle

The basic principle of the activated sludge process is that the wastewater is brought into contact with a mixed microbial population, in the form of a flocculent suspension, in an aerated and agitated system. Suspended and colloidal material are removed rapidly from the wastewater by adsorption and agglomeration on the microbial flocs. This material and dissolved nutrients are then broken down more slowly by microbial metabolism in a process referred to as 'stabilization'. In the stabilization process, part of the nutrient material is oxidized to simple substances such as carbon dioxide, a process known as 'mineralisation', and part is converted into new microbial cell material, a process known as 'assimilation'. Part of the microbial mass is broken down in the same way, as the organic matter and this process is termed as 'endogenous respiration'. The oxidative process provide the energy needed for the operation of the adsorption and assimilation processes.

When the desired degree of treatment has been achieved, the flocculent microbial mass, known as the 'sludge', is separated from the treated wastewater by gravity settling, usually in a separate, specially designed vessel. The separation stage is also referred to as 'clarifying', 'settling' or 'sedimentation'.

The supernatant from the separation stage should then be virtually free of sludge. A part of the settled sludge from the separation stage is returned to the aeration stage to maintain the sludge concentration in the aeration tank at the level needed for effective treatment and to act as a microbial inoculum.

The remaining sludge is removed for disposal, and is known as 'waste' or 'surplus' activated sludge. In a balanced system, the waste sludge produced represents the net amount of microbial mass produced by assimilation in the aeration stage, and is effectively the 'pollution concentrate' from the system.

The wastewater feed to the aeration tank will usually have passed through a primary treatment process, for removal of grit, oily and fatty material and gross solid material by physical methods such as settling and screening. The settled and cleared wastewater then passes to the aeration stage, so that the activated sludge process is often referred to as a 'secondary treatment'. However, certain versions of the activated sludge process are used without a primary treatment stage.

There is a variety of different versions of the activated sludge processes, from which arises its versatility in suiting a wide range of treatment requirements. They are made up of different combinations of modes of operation, mixing regime, aeration system and loading level. The essential features of the activated sludge process are an aeration stage, a separation stage and a sludge recycle system. The aeration systems available for use in the aeration stage can be divided broadly into bubble aeration, or 'diffuser', systems, or mechanical aeration systems, usually surface aerators, with sparged impeller, or 'combined' system containing elements of both.

2. 3 Importance of Microbial Evaluation in Wastewater Treatment

Without the action of microorganism, dead animals and plants would accumulate and choke the surface of the earth, (Mitchell, 1974). It is very important to know, in a wastewater treatment system, how microorganisms grow in mixed biological populations and what environmental factors affect their removal. Therefore, identification and enumeration of pathogenic or indicator microorganisms at different levels of treatment are required to get the information about the fate of these microorganisms present at different treatment levels. By following the standard identification procedures and isolatien techniques one can achieve this objective.

In wastewater treatment nearly all organisms contributing to substrate removal are welcomed and tend to be self-selecting. The bacteria, important in the aerobic treatment of sewage are rod-shape, facultative and mesophilic. They are excellent oxidizers of organic matter. Activated sludge treatment process, specially, is rich in these microorganisms. They are all capable of exuding a slimy flocculent layer which in Activated Sludge treatment units is an important mechanism in the treatment processes, (Duncan Mara, 1976). Geldreich et al. (1964) reported that, coliform and other intestinal bacteria do not play any significant role in the sewage treatment processes; they are merely passengers

Table. 2.2: Types and Numbers of Microorganisms Typically Found in Untreated Wastewater

ORGANISM	CONCENTRATION, (#/100 ml)
Total coliform	10^7 - 10^8
Fecal coliform	10^6 - 10^7
Enteric Virus	10^3 - 10^5
Clostridium Perfringens	10^3 - 10^5
Entrococci	10^4 - 10^5
Salmonella	10^2 - 10^4

After Feachem et al. (1983) & Mara, (1974).

in the system. Representative data on the type and number of microorganisms commonly found in wastewater are presented in Table 2.2.

2. 3 .1 Need of Indicator Microorganisms

The actual enumeration of pathogenic microorganisms that are present in the wastewater effluents is somewhat impossible due to the practical difficulties involved in their culture and handling. Their numbers in wastewater effluents are very low making their enumeration a time-consuming and complicated process. Therefore to avoid the health risks posed by the handling of pathogens and to provide a reasonable estimate of their numbers, indicator microorganisms are used (Cabelli, 1978).

In order to detect the pathogenic organisms in wastewater we use indicator organisms due to following main reasons (Metcalf and Eddy, 1991):

- Pathogenic organisms are small in number compared to indicator microorganisms and their detection is very difficult.
- From the safety consideration it is advisable not to work directly with pathogens.
- Reproduction rate of pathogens is usually slow and therefore more time is required for their detection.

Due to above reasons an ideal indicator might be considered to possess the following characteristics:

- It should present in wastewater and appear in polluted waters whenever pathogens are present.
- It should not be present in unpolluted waters.
- Indicators numbers should be greater in numbers than the pathogen.
- Indicator density should bear some relation to the degree or extent of pollution.
- Indicator microorganisms should survive longer than pathogens in waters and have equivalent resistance to disinfectants and other environmental conditions.
- It should be applicable to all types of water for which microbiological criteria serve a public health purpose.
- It should be detected by simple laboratory tests with a high degree of accuracy in the shortest possible time.

MetCalf, (1978) stated that no one indicator currently recognized meets all of these criteria, and it is doubtful if an ideal indicator exists or will ever be found for bacterial, protozoan, or viral pathogens.

Berg, (1973) suggested that for the complete microbial evaluation of the treatment facilities a group of indicators, of different type, be used.

2. 3 .2 Indicator Microorganism as an Evidence of Fecal Pollution

Coliforms are used as indicator organisms, as evidence of fecal pollution of water. The rationale behind this procedure is that *E.coli* is always present in sewage in high concentrations, approximately 10^6 per milliliter. In addition to this, its growth and survival is similar to pathogens. Fecal coliform, *Clostridium perfringens*, and coliphage are also good representatives of fecal pollution, (Tchobanoglous *et al.*, 1985).

Coliforms are considered as the indicator organisms of bacterial and viral pollution by many authors, (Morpis and Waite, 1980). The indicator coliform most often used is a group of microbes which are organisms normally found in the digestive tracts of warm blooded animals. Total coliforms are easy to detect and quantify. They are found in large numbers in polluted waters. It is estimated that a single person discharges an average of 1.95×10^9 coliforms per day. That is why coliforms are considered as the universal indicator organisms by some researcher (Geldreich, 1978).

Planter *et al.* (1990) reported a high degree of correlation between levels of coliphage and fecal coliforms in two highly polluted beaches in Scotland. It has been experimentally proven that coliphages will spread only wherever *E.coli* bacteria are found, and it is because of this fact, coliphages serve as pollution indicators.

Over many years *E.coli* was used as indicator for viral pollution. But there have been cases when epidemics have occurred even through the usage of coliform-free waters. Kott et al., (1974), suggested that coliphages might serve as better indicator of fecal pollution.

Borrego *et al.* (1986) developed a highly specific, sensitive and rapid technique for detection of coliphage. The numerical relationship between *E.coli* and its parasitic phages was investigated and the result of the study has indicated that coliphages are good indicators of the presence of pathogenic microorganisms.

Ratto *et al.* (1988) conducted a study in Lima, Peru, in which twenty samples were analyzed for coliphage content from five different sources. It was found that in 47% of the samples, coliphage were the only indicator organisms present. The presence of coliphage in these samples suggested that there is a high probability that human pathogenic virus can survive the conventional treatment processes.

Slade (1981), studied the occurrence, survival and pathogenic potential of viruses in sewage. He concluded that though it is highly unlikely, almost any virus could get into wastewater and survive treatment with unknown epidemiological consequences and stresses the need for studies that will help in the design and installation of appropriate treatment processes.

In a study conducted by Scarpino (1978), it was observed that the indigenous coliphages might be useful for evaluating the performance of

wastewater treatment plants in removing animal viruses. And since the bacteriophages (primarily the coliphages) provide a sensitive, convenient, economic and reliable index of water contamination by enteric viruses and bacteria, they have been proposed as an indirect measure of enteric virus presence in water and wastewater.

After studying the distribution of various pathogens with reference to the presence of indicator organisms, Geldreich (1978) has concluded that the prerequisites for the ideal indicator for fecal contamination in water has restricted the probable candidates to total coliforms. fecal coliforms, and *Clostridium perfringens.*

2. 4 Indicator Microorganisms Used in This Study

The indicator microorganisms used in this study were Standard Plate Ccunt, total coliform, fecal coliform, coliphage and *Clostridium perfringens.* Their specific characteristics and method of enumeration is described in the following sections

2. 4 .1 Standard Plate Count

Standard Plate Count enumeration is the simplest technique which provides an estimate of the density of aerobic and facultative aerobic heterotrophic bacteria in water. In this technique microorganisms are measured as colony forming units/ml on Standard Methods Agar plate after 48 hr. in

incubator at 35 °C as described in *Standard Methods for the Examination of Water and Wastewaters*, 16th Edition. APHA (1985). Standard plate count provides an indication of biological activity and facilitates the collection of reliable data for water quality control measurements, especially for comparative and legal purposes.

2. 4 .2 Total Coliform

The total coliform is a group which includes a broad spectrum of aerobic and facultative anaerobic, gram-negative, non-spore-forming bacilli that ferment lactose and produce gas within 48 hours at 35 °C. Some strains of coliform are widely distributed and are not specific to fecal material. Another coliform subgroup comprises of plant pathogens and other organisms of unknown taxonomy. All these coliform subgroups, however, may be found in sewage and in polluted water environment.

Total coliforms have long been recognized as suitable microbiological indicators of water quality largely because they are easy to detect and quantify. Coliform bacteria have been the traditional bacteriological tool for measuring the effectiveness of water treatment against fecal contamination. The average coliform density in feces from healthy individuals is $1.3x10^7$ per gram. It has been estimated by (Geldreich and Clarke, 1972) that 700 - 4500 PFU/100 ml or one virus per 20,000 - 65,000 coliforms are present in raw sewage. However many field investigations in polluted waters have revealed situations where total

coliform measurements cannot always be equated to the input of fecal wastes. In these instances, the nutrients present in raw sewage discharges can contribute biodegradable products that support the regrowth of some strains, thereby increasing the coliform to virus ratio. Some strains of coliforms are found in the natural environment and are not specific to fecal pollution. This factor combined with others makes it difficult to utilize total coliform as a realistic indicator of fecal pollution.

2. 4 .3 Fecal Coliform

The fecal coliform test measures *E.coli* and all other coliforms found in the intestinal tracts of warm-blooded animals intestinal tract that ferment lactose at 44.5°C. Fecal coliforms is a subgroup of the total coliform population, which corresponds more closely to the fecal contamination from warm blooded animals. The presence of this subgroup of the total coliform population is more accurately correlated with warm-blooded animals fecal discharges. In polluted waters, fecal coliform measurements relate more precisely to fecal contamination and are significantly less susceptible to bias caused by the regrowth characteristics of non-fecal coliforms. Geldreich (1966) reported that, about 93-98% of the total coliform group comprises of the fecal coliforms. However under excessive nutrient environments fecal coliforms too show a marked regrowth in polluted waters, (Ballentine and Kittrell, 1968). Data analyzed from numerous polluted streams indicates that fecal coliforms do not survive in

waters with a BOD of less than 30 mg/l. Enterovirus in sewage discharge were calculated to occurs at an approximate frequency of one PFU for every 100,000 fecal coliforms. Similarly one *Salmonella* per 540 fecal coliforms were reported in a creek sample taken from the sewage effluent outfall of a municipal secondary sewage treatment plant, (Cheng, e*t al.,* 1971).

2. 4 .4 Coliphages

Bacterial viruses that parasitize *E. coli* occur in the human gut and are believed to be present wherever *E. coli* are found. Bacterial viruses for which *E. coli* serves as a host cell are generally referred to as coliphages. The somatic coliphages comprises of all tailed and cubic bacteriophages capable of infecting a wide range *E.coli* host strain by adsorption to receptors in the cell envelope. Factors that favor a coliphage as an indicator are simplicity, economy, and rapidity of assay procedures. Scarpino (1978) concluded that the indigenous coliphages might be useful for evaluating the performance of the sewage treatment plants in removing animal viruses.

2. 4 .5 *Clostridium Perfringens*

Clostridium perfringens is used as a supplemental indicator, in addition to routine microbial examinations of water and wastewaters. It is an obligate anaerobe belonging to the sulfite-reducing spore forming group. *Clostridium perfringens* is a spore former and since such organisms can generally persist longer in water than non-spore forming bacteria such as coliforms, it has been

suggested that this anaerobe might be useful as an indicator of past pollution, (Wilson and Blari, 1925). This is usually done by comparing the *Clostridium perfringens* spores in a given sample with the unstressed recent vegetative cells. The vegetative cells are expected to predominate in the raw sewage. Spore development becomes responsive to the degree of sewage treatment, time and distance downstream from the point of discharge. Since bacterial spores are very resistant, *Clostridium perfringens* may also be used as an indicator of fecal pollution in waters receiving toxic industrial wastes that rapidly destroy other bacterial indicators. Fulton and Richardson (1971) reported that the methodology for the enumeration and detection of *Clostridium perfringens* is not complicated as it can tolerate upto 5% oxygen without significant loss of quantitative recovery. Tyrrell, et al. (1995) reported that *Clostridium perfringens* are highly refractory to ozone and chlorine disinfectants.

Buras (1974), Bevins and Sproul (1967), concluded that, where a rigorous test of sewage treatment is desired, including sewage control, a limit on *Clostridium perfringens* densities in the discharge can prove more meaningful than the traditional coliform standards.

2. 5 Fate of Pathogenic Microorganisms Present in Domestic Wastewater

Pathogenic organisms found in wastewater may be discharged by human beings who are infected with disease or who are carriers of a particular disease.

The principal categories of pathogenic organisms found in wastewater are bacteria, viruses, protozoa, helminths, etc. A list of pathogenic microorganisms is presented in Table 2.3. The usual bacterial pathogens that may be excreted by humans cause diseases of the gastrointestinal tract such as gastroenteritis, dysentery, diarrhea, and cholera.

Geldreich et al. (1962) reported a range of 10^6 to 10^9 fecal coliform released per gram of human feces, and for pet animals ranges from 10^4 to 10^7 per gram. Another group of these organisms are viruses. Over a hundred viruses can be found in raw sewage, (Goddard & Butlers, 1980). Viruses may cause infectious hepatitis, epidemic gastroenteritis and epidemic diarrhea. Because these organisms are highly infectious, they are responsible for thousands of deaths each years in areas with poor sanitation, especially in the tropics (Feachem *et al.*, 1983 and Mara *et al.*, 1974).Viruses that are excreted by human beings may become a major hazard to public health. For example from experimental studies, it has been found that from 10^4 to 10^5 infectious doses of hepatitis virus are emitted per gram of feces of an infected patient (Haenel and Muller, 1956).

The viruses infecting humans are the most important group, at least from the point of view of human health, and are found mainly in sewage from domestic sources. A major subgroup is the fecally excreted enteric viruses and these are the most widely studied in the context of wastewater disposal and water supply.

Table.2.3: Infectious Agents Potentially Present in Domestic Wastewater

ORGANISM	DISEASE	REMARKS
Bacteria		
Escherichia Coli (entropathogenic)	Gastroenterities	Diarrhea
Legionella pneumophila	Legionellosis	Acute respiratory illness
laptospira (150 spp.)	Leptospirosis	Jaundice, fever (Weil's disease)
Salmonella typhi	Typhoid fever	High fever, diarrhea, ulceration of intestine
Salmonella (~1700 sp.)	Salmonellosis	Food poisoning
Shigella (4 spp)	Shigellosis	Bacillary dysentery
Vibrio cholerae	Cholera	Diarrhea, dehydration
Yersinia enterolitica	Yersinosis	Diarrhea
Viruses		
Adenovirus	Respiratory disease	
Enteroviruses (67 type)	Gastroenteritis, heart anomalies, meningitis	
Hepatitis A	Infectious hepatitis	Jaundice. Fever
Norwalk agent	Gastroenteritis	Vomiting
Reovirus	Gastroenteritis	
Rotavirus	Gastroenteritis	
Protozoa		
Balantidium coli	Balantidiasis	Diarrhea, dysentery
Cryptosporidium	Cryptosporidiosis	Diarrhea
Entamoeba hystolytica	Amebiasis (amoebic dysentery)	Prolonged diarrhea with bleeding, abscesses
Giardia lamblia	Giardiasis	Mild to severe diarrhea, nauses, indigestion
Helminths		
Ascaris lumbricoides	Ascariasis	Roundworm infestation
Enterobius vericularis	Enterobiasis	Pinworm
Fasciola hepatica	Fascioliasis	Sheep liver fluke

Adopted from Feachem et al. (1983) and Stanier at al. (1986).

Evaluation of the relative risk of disease transmission associated with land application of wastewater requires knowledge of the number of pathogens in untreated and treated wastewater, as well as the number necessary to cause an infection in man or other animals. Unfortunately, data on the removal efficiency of all wastewater treatment methods for many pathogens are either nonexistent or largely based on laboratory studies by researchers who may overestimate the efficiency that can be obtained in actual practice (Foster and Engelbrecht, 1973). From currently available information, Foster and Engelbrecht, (1973) attempted to estimate the relative concentrations of pathogens in untreated wastewater and the relative efficiency of removal by primary and secondary treatment. The estimated concentration of wastewater pathogens in treated and untreated effluents of a sewage treatment plant is shown in Table 2.4.

2. 6 Fate of Indicator Microorganisms in Wastewater Treatment Plant Effluents

Sewage treatment processes can reduce the pathogen content of raw sewage. The reduction is governed by length of retention time during treatment, chemical composition of the wastes and their state of degradation. Antagonistic forces in the biological flora, pH, and operational temperature are among the more subtle and less understood factors.

Table. 2.4: Estimated Concentrations of Wastewater Pathogens in Treated and Untreated Effluents of a Sludge Treatment Plant.

PATHOGEN	NUMBER OF ORGANISMS PER GALLON			
	Untreated Wastewater	Primary Effluent	Secondary effluent	*Chlorinated effluent
Human enterovirus	4.0×10^4	2.0×10^4	2.0×10^3	2.0×10^2
Salmonella	2.0×10^4	1.0×10^4	5.0×10^2	5.0×10^{-1}
E. Histolytica	1.5×10^1	1.3×10^1	1.2×10^1	1.2×10^{-2}
Helminth Ova	2.5×10^2	2.5×10^1	5.0×10^0	5.0×10^{-3}
Mycobacterium	2.0×10^2	1.0×10^2	1.5×10^1	1.5×10^{-2}

After Foster and Engelbrecht, (1973).

* Conditions sufficient to yield a 99% kill.

Data available in the literature indicates that pathogenic microorganisms, particularly viruses, pass through the sewage treatment processes in large numbers. Gilcreas *et al.* (1954), reported the reduction of total coliform in activated sludge system ranged from 82 to 97%. Similar values, for total coliform removal has been reported by McCoy (1970). Cram (1943) reported a reduction of 85 to 98% of total coliform, *Salmonella*, *Shigella*, and *M. tuberculosis* in an activated sludge treatment system. Lance, J. C. (1983) reported that approximately 76 to 99% removal of coliform can be achieved in activated sludge treatment process.

Omura et al. (1989) carried out a study on removal efficiencies of indicator microorganisms in different Sludge Treatment Plants. Results obtained for a conventional activated Sludge Treatment Plants is presented in Table 2.5, showing a removal efficiency of bacteria up to 97% in secondary treated effluent and viruses up to 96.6%. Another study carried out by Rao et al. (1974), on virus removal efficiency in a conventional activated STP reported a removal of 90 to 99.1%.

After an extensive laboratory study Clarke et al. (1961) reported a fecal coliform reduction of 96 to 99.4% in secondary effluent of an activated sludge treatment plant. Essentially no removal of enteric viruses is accomplished by primary treatment. Various investigations have been undertaken to measure the level of coliphage in wastewater systems and reported a tenfold reduction in numbers after two hours of aeration in secondary treatment.

Table. 2.5: Microbial Removal Efficiency of Conventional Activated Sludge Treatment Plant.

MICROORGANISM	CONVENTIONAL ACTIVATED SLUDGE TREATMENT PLANT	
	Secondary effluent	Chlorinated effluent
Total coliform	91.60	99.9999
Fecal Streptococcus	97.00	99.99
Coliphage	96.60	99.99

After Omura et al., (1989).

Durham and Wolf(1973), reported a 99.13% reduction of total coliform and 95.5% reduction of coliphage in an activated sludge treatment plant after secondary treatment.

Even under proper operating conditions, the resulting treated effluents will still contain a portion of each kind of microorganism originally present in the raw sewage. These pathogenic bacteria, virus, and parasites constitute a potential hazard to persons coming in contact with the receiving waters. The presence of pathogenic/indicator microorganism has a great importance from community health and safety point of view. Hence, sewage should be treated before its ultimate disposal in a receiving watercourse or for reuse in order to

a) reduce the spread of communicable diseases caused by pathogenic organisms in the sewage and,

ii) prevent the pollution of surface and ground waters.

2. 7 Fate of Indicator Microorganisms in Chlorinated Effluents

In a research carried out by Omura et al. (1989) on an activated sludge treatment plant, having residual chlorine range (0-1.52 mg/l), zero coliform after chlorination and about 99% reduction in virus concentration was reported. Another study on Damman sewage treatment plant, carried out by Farooq et al. (1997), reported a 98.9% reduction of total coliform during winter after chlorination and 98.48% reduction during summer, while residual chlorine was

absent most of the time. The average value of total coliform present in the chlorinated effluent was reported as 1,075 MPN/100 ml and 6,480MPN/100 ml during winter and summer respectively. Engelbrecht et al. (1974), found no coliform in chlorinated effluents during a study conducted at Urbana to find a new indicator of chlorine efficiency.

Butler (1981), studied the mechanisms of virus removal by various disinfection processes and he regarded conventional wastewater treatment processes as highly inefficient and stressed the need for tertiary treatment and disinfection as a means of a more comprehensive virus removal.

In a study of the chlorination experiments on f2 and MS_2 coliphages, attenuated with Polio I strain, Kott *et al.* (1974), have reported that the coliphages were more resistant than the attenuated Polio I virus. This study establishes that bacteriophages, particularly coliphages can serve as viral pollution indicators in wastewater treatment.

Havelaar & Nieuwstad, (1985) in a study established that to achieve a 3 log unit kill of bacteriophages a chlorine dose of 16 mg/l is necessary.

A study conducted by Borrego *et al.* (1986) to test the feasibility of *E. coli* specific bacteriophages as universal fecal pollution indicators has indicated that coliphages are good indicators of the presence of pathogenic micro-organisms. It is claimed that coliphages performed better as indicators of fecal pollution than the classical indicator systems currently employed.

Clostridium perfringens is found in the normal intestinal flora of man and animal (Akama, 1970; Bisson and Cabelli, 1980), and excreted with their feces to the environment. Furthermore, this bacterium is not capable of proliferating in the environmental waters and/or soils (Boyd et al., 1948; Fuchs et al., 1957). The field study on the incidence (Hirata et al., 1986) suggests that the *Clostridium perfringens* has a high ability to survive in environmental waters compared to the others indicator microorganisms.

In a study carried out by Imran, (1997) on removal of microorganisms by the application of a chlorine dose of 5mg/l to secondary effluents, reported a removal of 87.3% in Standard Plate Counts, 76.6% in total coliform, 84.9% in fecal coliform, 44.5% in *Clostridium perfringens* and 49.1% in coliphage. In the same study, by applying a 15mg/l chlorine dose, he reported a removal of 99.74% in Standard Plate Counts, 99.93% in total coliform, 99.95% in fecal coliform, 71.35% in *Clostridium perfringens* and 70.6% in coliphage.

MetCalf & Eddy (1991), and Tyrrell et al. (1995) reported that the *Clostridium perfringens* (spore-forming anaerobic persistent bacteria) is a desirable indicator where disinfection is employed, where pollution may have occurred in the past, or where the interval before analysis is projected.

Hirata et al., (1991), recommended the *Clostridium perfringens* to be an effective and practical microbial tracer of fecal pollution through its high resistance to chlorine, high conservation in environment, and lower removal in wastewater and sludge treatment compared to other indicator microorganisms.

2. 8 Fate of Indicator Microorganisms in the Sludge

Most of the viruses and bacteria in sewage are sedimented with the solids that settle as sludges in primary settling basins and in the activated sludge and chemical sludge basins of secondary and tertiary treatment processes. Although animal viruses cannot multiply in such sludges, bacteria can; thus, even if a given ratio of some indicator bacteria to viruses existed, after a residence period in the sludges, that ratio is likely to be distorted. Toxic material in sludges, effecting viruses and bacteria differently, could also affect these ratios (Berg and MetCalf, 1978).

A study carried out by Pepper, et al. (1993), over a period of 23 months on survival of indicator microorganisms in soil amended with sewage sludge. The site chosen for the study was the Southwestern USA, which has an arid climate. In this study the authors monitored total coliform, fecal coliform and fecal streptococcus (utilized as indicator microorganisms due to the presence of higher concentration in sludges) and reported concentrations of these microorganisms of 10^{11} , 10^{10} and 10^{9} per liter. They reported decreasing pattern of microbial population with the increase in temperature. They also reported the survival time of these microorganisms as

fecal streptococcus > fecal coliform > total coliform

Fecal coliform required atleast 84 days for complete inactivation at a temperature of 20 to 25 °C. This study confirms the findings of Yeager and

Ward (1981), who identified the importance of moisture for regrowth of microorganisms in sewage sludge.

A study by Berg et al.(1976), over a period of 18 months showed that, the number of viruses recovered from raw sludges ranged from 380 PFU to 11,600 PFU per 100 ml of sludge. The ratios of fecal coliform to viruses ranged from 3,300:1 to 970,000:1. The ratios of total coliform to viruses were found to range from 34,000:1 to 13,000,000:1, and the ratios of fecal streptococci to viruses ranged from 603:1 to 95,000:1.

Drying of sludges at 35°C for about 20 days destroyed 76.0 to 96.0% of the viruses, 95.0 to 99.3% of the fecal coliform, 86.0 to 99.5% of the total coliform, and 80.0 to 97.0% of the fecal streptococci, (Berg et al., 1976).

The extended survival of pathogens in dry sludge could be eliminated by application of higher temperatures during storage prior to disposal. Treatment with sufficient lime to raise the pH above 11 would also achieve good inactivation of viruses. Sorber et al., (1984) reported the elimination of microbial activity in the de-watered sludge cake when mixed with a bulking agent for 2 to 10 weeks period. Gaudy & Gaudy (1980) reported that anaerobically digested sludge inactivates several enteric viruses, but Kollins, (1966) reported the presence of active viruses in the drainage from sludge drying beds after two weeks. Wellings et al., (1976) recovered viruses from caked sludge that had been exposed to hot sun for 13 days on a drying bed.

In the WPCF report Sorber et al., (1984) stated as, there are many sludge disinfection processes available to the design engineer. However, there is no universally accepted process which is ideal for most locations, as there is for wastewater disinfection. The paucity of available literature on this subject indicates that more research and development must be done before the state-of-the-art technology in sludge disinfection rivals that of water or wastewater disinfection. Regardless of the lack of available literature, the ultimate responsibility for the protection of the public welfare from health hazards arising from the application of municipal sludge to land remains with the engineer and the user agency.

2. 9 Growth and Survival of Microorganisms

Microorganisms assimilate waste material and utilize them as energy sources and as building blocks for microbial growth. In the process a new population is developed and catabolites, or degradation products, are excreted, (Mitchell, 1974).

Bacterial populations attain high densities very rapidly. A rapid population explosion is produced exponentially. *E. coli* divides approximately every 20 minutes. Inoculation of 1000 cells of *E. coli* to a nutrient medium will yield more than 1 million cell within 6 hours.

Not all bacteria have the same generation time; for some, such as *Escherichia coli*, it may be 15 to 20 min.; for others it may be several hours as

shown in Table 2.6. Similarly, the generation time is not the same for a particular bacterium under all conditions. The generation time is strongly dependent upon the nutrients in the medium and on prevailing physical conditions like temperature, gaseous requirement, pH, oxidation reduction potential, etc.

2. 9 .1 Effect of Temperature on Microbial Population

As all the processes of growth are dependent on chemical reactions and the rate of these reactions are influenced by temperature, the pattern of bacterial growth can be profoundly influenced by these conditions. Temperature in part, determines the rate of growth and the total amount of growth as well as the metabolism and morphology of the organism.

Michael *J. et al.* (1976) stated that each species of bacteria grows at temperatures within a certain range. The approximate temperature range for growth of various bacteria is presented in Table 2.7. On this basis bacteria are divided into the following groups:

1. Psychrophiles are able to grow at 0°C or lower, though they grow best at higher temperatures, closer to 15 or 20 °C. The effect of temperature on the growth of a psychrophilic Bacillus sp. is shown in Table 2.8.
2. Mesophiles grow best within a temperature range of approximately 25 to 40°C. Mostly microorganisms important in the function of biological wastewater treatment are mesophiles.

Table. 2.6: Generation Time of Several Species of Bacteria

BACTERIUM	MEDIUM	OPTIMUM TEMPERATURE (°C)	GENERATION TIME, MIN.
Escherichia Coli	Broth	37	17
Escherichia Coli	Milk	37	12.5
Bacillus mycoides	Broth	37	28
Lactobacillus acidophilus	Milk	37	66-87
Mycobacterium tuberculosis	Synthetic	37	792-932
Treponema pallidum	Rabbit testes	37	1980

Source: Data from W. B. Spector (ed.), "Handbook of Biological Data," W. B. Company philadelphia, 1956.

Table. 2.7: Approximate Temperature Range for Growth of Various Bacteria

BACTERIA GROUP	TEMPERATURE RANGE OF GROWTH (°C)
Psychrophiles	-5 - 35
Mesophiles	15 - 45
Facultative thermophiles	25 - 55
Thermophiles	40 - 75

After Michael J. et al. (1976).

Table. 2.8: Effect of Temperature on the Growth of Psychrophilic Bacillus sp.

CULTIVATION TEMPERATURE (°C)	GENERATION TIME (hr.)
25.0	2.5
20.0	2.5
15.0	3.0
10.0	6.0
5.0	8.5
0.0	23.0

After Stokes, (1968)

The temperature of incubation which allows for most rapid growth during a short period of time (12 to 24 hr) is known as optimum-growth temperature. The most favorable temperature for pathogenic bacteria is human body temperature, which is 37°C, (Kott et al., 1974). It is reported that survival of pathogens is enhanced by lowering the temperature. Gaudy & Gaudy, (1980) have reported the survival of enteric viruses for 9 to 10 months in wastewater at 8°C.

Barzily and Kott (1991), in a study found that the die-off of total coliform, fecal coliform, and fecal streptococcus were directly related with the increase in temperature. However, up to 40 °C the phage survived was higher (73.73%) as compared to the bacterial indicators.

2. 9 .2 Effect of Wastewater Flowrate on Microbial Population

Variation of bacterial population has been measured by MetCalf and Eddy, (1991) in sewer systems. They reported a lower concentration of microorganisms during high wastewater flowrate. When flowrate decrease the concentration of microorganism rise significantly. The decrease in the population density of microorganism could be justified due to the dilution effect of the water during high rate of water utilization season. Another study carried out by El-Sharkawi et al. (1989), reported the similar results from their study.

2. 10 Treatment Efficiency of a Wastewater Treatment Plant

Treatment efficiency of many sewage-treatment processes is almost entirely dependent upon microbial growth and metabolism. The chemical activities of the microorganisms are responsible, to a major degree, for stabilization of the final effluent.

Gainey & Lord (1952), have noted that the efficiency reported for any single process shows considerable variation. This may be attributed to the design of the unit, the method of operation, the nature of sewage, or other differences. The efficiency of wastewater treatment obtained by the several procedures is shown in Table 2.9

2. 11 Scope of the Study

After carried out, a detailed literature survey it was found that there are some areas in which more research is required. Historically, research on wastewater treatment processes, performance of treatment plant and quality of wastewater is going on since last century (Purdy and Butterfield, 1918). Much attention is required in some areas in which little information and data is available.

There is a need to see the trend of different microorganism population densities with the seasonal temperature and flowrate of wastewater to

Table. 2.9: Efficiency of Various Sewage Treatment Methods.

METHOD OF TREATMENT	PERCENTAGE OF REMOVAL	
	Bacteria	BOD
Plain sedimentation	40-75	30-35
Chemical precipitation	80-90	60-80
Septic tank	40-75	25-65
Imhoff tank	40-75	25-65
Intermittent sand filters	98-99+	70-96
Contact bed	50-75	60-80
Trickling filter	70-85	60-90
Activated sludge	95-99+	70-96

After Gainey & Lord, (1952).

understand the growth and survival of these microorganisms more clearly with these factors. Study on dry sludge area is in progress and increase in the use of dry sludge as fertilizer in some communities increases the importance of this study. As health and safety problems involved in the handling of dry sludge due to the presence of large number of pathogen in it, more research in this area is required. The paucity of available literature and data on this subject is also reported by some researchers (Sorber et al., 1984).

In order to evaluate the Al-Khobar wastewater treatment plant from microbial point of view it is necessary to find the removal efficiencies of different microorganisms after different treatment to see the behavior of these microorganisms against treatment.

CHAPTER 3

3. OBJECTIVES

Microbial population densities, temperature and flowrate have been measured on a weekly basis in raw sewage and other effluents of the treatment plant over a period of one year. The following were the specific objectives of this study.

1. To study the effect of seasonal variations of temperature and flow rate on the fate of five indicator microorganisms in different effluents of the wastewater treatment plant.
2. To study the effect of seasonal temperature variations on the fate of studied indicator microorganisms in the dry sludge of the treatment plant.
3. To assess the effect of chlorination on different indicator microorganisms.
4. To assess the removal of each of the studied indicator microorganism in different effluents of the treatment plant.

CHAPTER 4

4. MATERIALS AND METHODS

The objectives of this study were achieved by an intensive experimental program that lasted over a period of one full year.

Initially a background study/survey had been done. This phase was accomplished by frequent visits to the Al-Khobar sewage treatment plant. History of the plant, design criterion and any transient occurrence at the plant had been studied. This part of the study acted as a foundation for this thesis work.

The second phase consisted of sampling and laboratory experiments on weekly basis. This extensive data collection phase included the summer and winter seasons. In the final stage, analysis of data and preparation of thesis write-up has been done.

4. 1 Indicator Microorganisms Studied.

The most widely used indicator microbial parameter for pollution evaluation, to both wastewater and surface water are coliform and fecal coliform. In this study in addition to these microorganisms some other microorganisms have also been studied.

Standard Plate Counts were determined to get an idea of overall concentration of microorganisms in different effluents of the treatment plant. total coliform, fecal coliform, and coliphage enumerated due to their importance in the wastewater treatment as fecal pollution indicators (described in sec 3.4). *Clostridium perfringens* group (spore forming) has been used, because it has high resistance against high temperature and chlorine disinfection.

4. 2 Sampling Technique and Sampling Points.

Proper sampling techniques are vital for accurate testing in evaluation studies. An instantaneous grab sampling was done for microbial sample analysis. Weekly samples were obtained from the following sampling points

Type of sample	**Sampling Point**
Influent	Inlet structure
Secondary effluent	Outlet of secondary clarifier
Chlorinated effluent	Outlet of chlorination chamber
Dry sludge	Sludge drying beds

Liquid samples were collected in clean 300 ml glass bottles. Sodium thiosulfate was used in chlorinated sample collection bottle in order to neutralize the effect of residual chlorine. Dry sludge sample were collected from one of the sludge drying bed, located inside the boundary of the Al-Khobar sewage treatment plant. In order to get the representative sample, dry sludge samples were collected from the depth of 5 to 10 cm from the surface of the bed. Dry sludge samples were collected in petridishes and immediately covered with a polythene sheet. After collection samples were transferred immediately to the lab in ice boxes. Strict sterilized conditions were maintained throughout collection and transportation of these samples.

4. 3 Equipment and Media Used.

Microbial analysis of samples includes detection and enumeration of the parameters: Standard Plate Counts, total coliforms, fecal coliforms, *Clostridium perfringens*, and coliphages. The analysis for all the parameters have been carried out weekly to monitor the microbial population density of the indicator microorganisms.

All the equipment, media, and facilities for the microbial analysis are available in the Environmental Engineering Laboratory at KFUPM. Optimum sterile conditions were maintained to protect staff from exposure to pathogens and for the handling of the microbial samples and culture media.

Different laboratory equipment were used routinely for microbial analysis. Autoclave was used to sterilize the media and other related materials. Incubator was used for inoculated culture growth. Heating water baths were used for *Clostridium perfringens* isolation. Similarly colony counter, pH meter and many other equipment were used in the laboratory.

Media, defined in *Standard Methods for Water and Wastewater Analysis* (1985) had been used for the isolation of different microorganisms. A summary of microbial parameters, the method of detection and enumeration and culture media used are presented in Table 4.1.

4. 4 Microbial Culture Techniques.

The analysis of microbiological parameters, i.e., Standard Plate Counts, total coliform, fecal coliforms, *Clostridium perfringens* and coliphage were carried out as prescribed in *Standard Methods* (1985). The bacterial density of Standard Plate Count in wastewater was determined using Spread Plate (Section 907B) technique. Multiple tube technique (Section 908A, C) was used for the detection of total coliforms and fecal coliforms. *Escherichia coli* C, ATCC No. 13706 was used as host culture for the detection of coliphage (Section 919C). Dilution of samples were made wherever found necessary, as per the requirement of application of procedures in *Standard Methods* (1985).

Table. 4.1: Culture Media Used for Detection and Enumeration of Indicator Microorganisms

Indicator Microorganisms	Method of Detection and Enumeration	Culture Media	Incubation Time/Temperature
Standard Plate Counts	Pour plate technique	Standard Method Agar	48 hr. (35.5°C)
Total Coliforms	MPN method	Lactose broth and BGB broth	48 hr. (35.5°C)
Fecal Coliforms	MPN method	EC broth	24 hr. (44.5°C)
Cl. perfringens	*Membrane filter technique	Enriched Clostridial Agar	24 hr. (37°C)
Coliphages	Plaque forming units in a lawn of E. coli host cells	Tryptic Soy Agar, Tryptic Soy Broth, and Modified Tryptic Soy Agar.	24 hr. (35.5°C)

Source: Standard Methods, 16th Ed.(1985).

* Mackie and McCartney (1989).

Spore forming anaerobic *Clostridium* are present in smaller numbers and are estimated by using a membrane filter technique (Mackie and McCartney, 1989).

The sample was heated at 75°C for 10 minutes to kill all vegetative bacteria. A sterile filtration unit is mounted with 0.45 µ size membrane. A sample of 25 ml is taken and pressure for filtration is turned on. The membrane is then asceptically transferred to an sterile petridish.

One ml sodium sulfite (10%) and 0.1 ml ferrous sulfate (8%) are added in the melted basal agar media (modified from Burman *et al.*, 1969), and immediately poured over the petridish containing the membrane. The dishes are placed in an air tight container to prevent any air penetrating into it and incubated at 37°C for 48 hours. After incubation *Cl. perfringens* colonies characterized by circular black agglomerations, are counted and the concentration is expressed in 100 ml of the sample.

The percent solids in the dry sludge were determined on a dry weight basis by drying at 103 to 107 °C in an oven for 24 hrs. and found to be 2 to 15%. For microbial analysis ten fold dilutions starting with 1 ml of thoroughly mixed sludge and 9 ml of a 0.1% peptone solution were assayed in triplicate.

Both the total coliform and fecal coliform were enumerated by Multiple Tube Fermentation technique and the results expressed as MPN (Most Probable Number) per 100 ml of sample. For Standard Plate Count, and *Clostridium perfringens* results were reported as colonies per 100 ml while coliphage,

reported as PFU (Plague Forming Units) per 100 ml of sample. Results of dry sludge analysis were reported as per gram of sludge.

4. 5 Statistical Analysis

To drive meaningful conclusions about the microbial population densities, the data were statistically analyzed.

In order to determine whether there exists any significant difference between the means of microbial population densities noted during summer and winter seasons, student t-test were used to determine the significant difference between the two.

4. 5 .1 T-test Analysis

Much of the research in engineering and science makes use of statistical analysis to greatly increase the efficiency of the experiment and strengthen the combination of data sets. Statistical analysis refers to the process of planning various combinations in order to know weather observed difference in the data have any statistical significant difference or not.

The t-test can be performed assuming equal variances in the two data sets or by assuming unequal variances. Since it was not clear whether the variances in the two sets were equal or not, the t-test was performed by assuming unequal variances. In the event that the variances were equal, the t-test assuming unequal variances automatically reduces to a t-test assuming equal variances. To test the above hypothesis, the t-test assuming unequal

variances was performed using the *Microsoft Excel* package. The calculated value is compared with the t-critical value at a confidence level of 95 percent.

The criteria of the good fit of the data is coefficient of determination (R^2) which should be closer to unity '1' in case of good fit and when its value approaches to zero '0' the data lack in fit.

4. 5 .2 Regression Analysis

In most of the problems there are two or more variables that are related, and it is important to model and explore the relation. Regression methods are frequently used to analyze the data from unplanned experiments and this analysis is also very useful in designed experiments. Generally, the Analysis of Variance in a designed experiment helps to identify which factors are important and regression is used to build a quantitative model relating the important features to response. After we have decided which distribution to use in describing the data, we must estimate the parameters appearing in the distribution. In this study most important characteristic parameter is mean value to whom we are making the bench mark for the subsequent analysis.

In order to relate the flow and temperature during different seasons with the microbial population density in different effluents of the treatment plant Regression Analysis were used for Modeling as a tool. After many trials Microsoft Excel-7 and Sigma Stat computer packages were used to execute multiple non-linear stepwise regression analysis.

4. 5 .3 Trials For Analysis

To develop the relationships first simple multiple linear regression model were tried as given in Eq. 4.1

$$Y = \alpha_o + \alpha_1 T + \alpha_2 F \qquad (4.1)$$

In this model the T and F are the wastewater temperature and flow rates and Y is the response or dependent variable (microbial population density). Similarly other models like equations 4.2, and 4.3 were tried to get suitable models.

$$Y = \alpha_o + \alpha_1 T^2 + \alpha_2 F^2 \qquad (4.2)$$

$$\ln(Y) = \alpha_o + \alpha_1 \ln(T) + \alpha_{2l} \ln(F) \qquad (4.3)$$

After many trials finally a general regression equation was developed.

CHAPTER 5

5. RESULTS AND DISCUSSION

The microbial parameters of environmental concern for Al-Khobar wastewater treatment plant were analyzed on weekly basis. The Environmental Engineering laboratory at KFUPM was used for the detection and enumeration experiments for different indicator microorganisms.

Sampling and laboratory analysis were carried out over a period of 12 months, based on the methodology outlined in Standard Methods for Water and Wastewater Analysis (1985). The first sample was collected and analyzed during the first week of March, 1996 and the last analysis was done in the last week of April, 1997.

A detailed data analysis for the Standard Plate Counts, total coliform, fecal coliform, coliphage, and *Clostridium perfringens* population densities were performed for the summer and winter seasons. After the overall analysis, data were analyzed logically and statistically, specifically for different wastewater effluents of the plant and the dry sludge.

Generally there was an appreciable difference observed in the data obtained during the summer and winter seasons.

5. 1 Temperature and Flow Variations at The Al-Khobar Wastewater Treatment Plant

Variation of wastewater temperature with the time or season change at the Al-Khobar sewage treatment plant is presented in Fig 5.1. Similarly variation of flow during this year is shown in Fig 5.2. The wastewater flow data was fitted to different candidate distributions like Normal distribution, Log normal distribution and Weibull distribution to get the representative value. The Log normal distribution was find to be the best fit to the wastewater flow data. Log normal distribution which is a skewed distribution takes care of the variation of magnitudes in the data (Bedient, and Huber, 1992). The plots were used to determine the mean value, the median value, the 95 percent flow and standard deviation in the data. The mean was determined to note the arithmetic average value, the median to see if the distribution was symmetrical, the standard deviation to see the absolute dispersion of the set of flow and temperature measurements (Techobenoglus et al.,1993).

The incoming flow at the treatment plant ranged from 124,880 to 82,050 m^3/day with an average value of 97,928 m^3/day. The most frequent (mode) flow was 91,210 m^3/day and the median value was 97,538 m^3/day. The

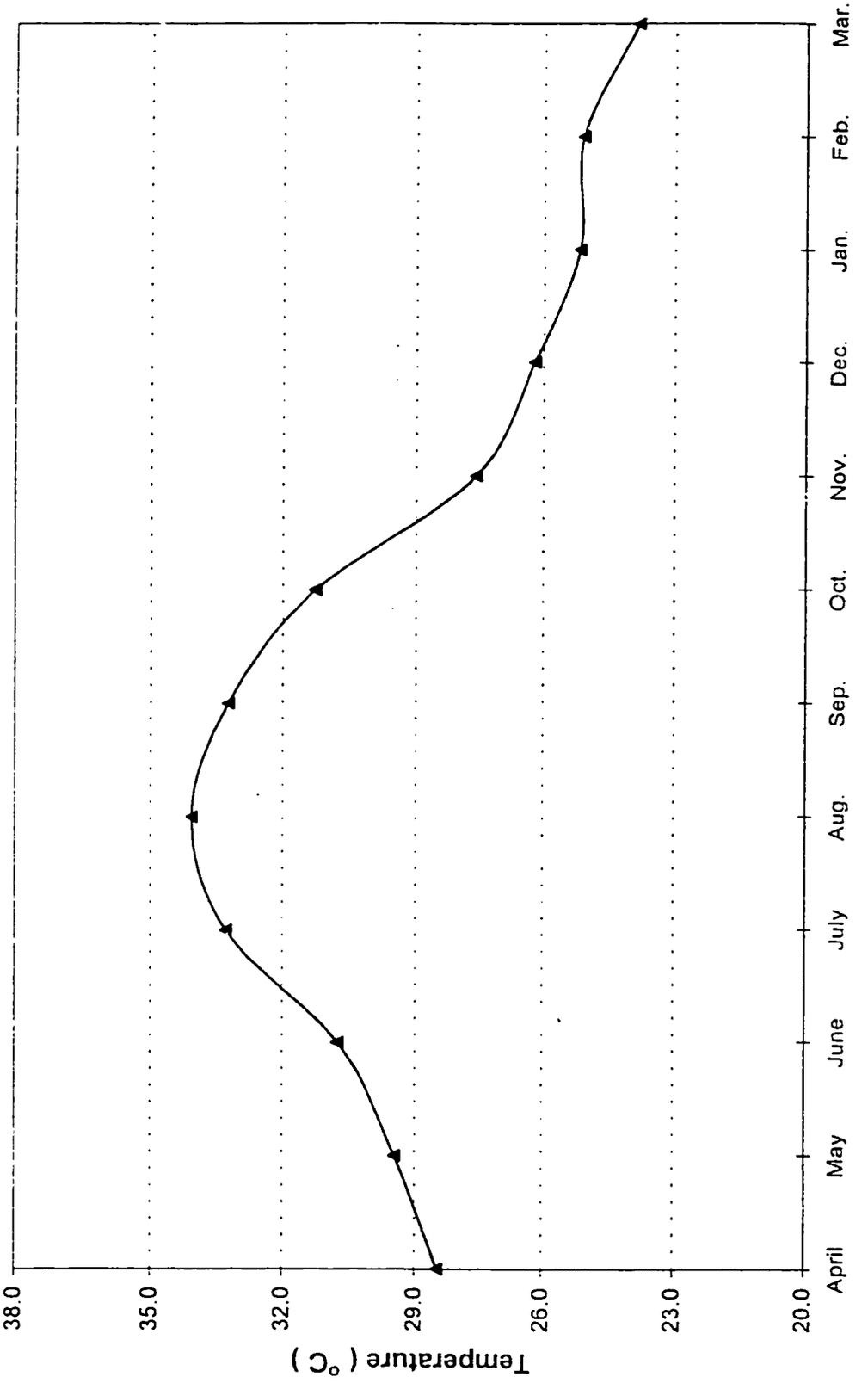


Fig. 5.1: Variation of Wastewater Temperature at Al-Khobar STP

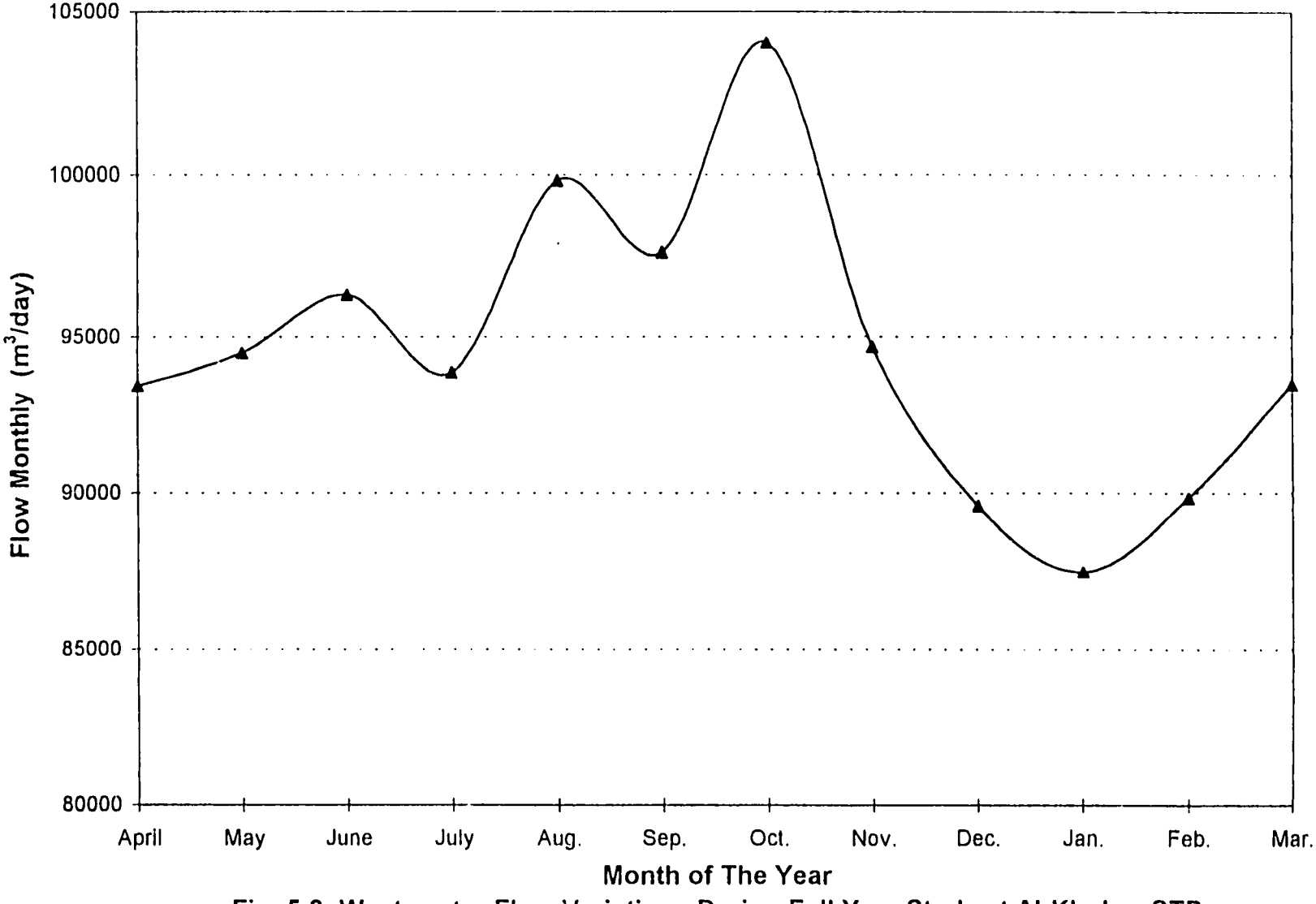


Fig. 5.2: Wastewater Flow Variations During Full Year Study at Al-Khobar STP

standard deviation was 8,762 m^3/day. The 95 percent flow was determined from the log normal distribution plot as 109,398 m^3/day (Ramakumar 1993).

Maximum and minimum temperature of the wastewater in the treatment plant ranged from 34.6 to 23.5 °C with an average of 29.1 °C. Since variation in the temperature data was not significant, a simple average value was calculated.

5. 2 Microbial Population Density Observed During One Year Study.

A detailed data analysis was carried out on the population densities of each indicator microorganism, in different effluents of the Al-Khobar wastewater treatment plant. The full year average population density in the influent was $3.48x10^7$ MPN/100 ml and $1.61x10^7$ MPN/100 ml for total coliform and fecal coliform and $4.71x10^{10}$ colonies/100 ml, $8.26x10^2$ PFU/100 ml, and $7.51x10^2$ colonies/100 ml for Standard Plate Count, coliphage, and *Cl. perfringens* respectively. Population density data was statistically analyzed and tested for different distributions. The log normal distribution which was found give the best fit was selected. The values of range, mean, median, and standard deviation for each microorganism in different effluents and dry sludge of the treatment plant are presented in Table 5.1.

Table 5.1: Summary of the Results Obtained Over a Period of One Year

Sample		SPC	TC	FC	CP	Cl.Pr
Raw	Max.	$1.01x10^{11}$	$9.80x10^{7}$	$2.93x10^{7}$	$2.80x10^{3}$	$1.93x10^{3}$
Sewage	Min.	$3.86x10^{8}$	$5.85x10^{5}$	$9.05x10^{4}$	$1.00x10^{2}$	$1.70x10^{2}$
	Mean	$4.71x10^{10}$	$3.48x10^{7}$	$1.61x10^{7}$	$8.26x10^{2}$	$7.51x10^{2}$
	Median	$6.04x10^{9}$	$7.87x10^{6}$	$2.84x10^{6}$	$4.12x10^{2}$	$5.33x10^{2}$
	Std.Dev.	$3.64x10^{10}$	$3.57x10^{7}$	$1.15x10^{7}$	$9.94x10^{2}$	$5.92x10^{2}$
Secondary	Max.	$1.82x10^{8}$	$9.93x10^{5}$	$2.50x10^{6}$	$1.80x10^{2}$	$4.52x10^{2}$
Effluent	Min.	$1.02x10^{6}$	$1.42x10^{4}$	$1.01x10^{4}$	$4.50x10^{1}$	$1.70x10^{2}$
	Mean	$7.16x10^{7}$	$2.70x10^{5}$	$1.97x10^{5}$	$1.35x10^{2}$	$3.34x10^{2}$
	Median	$2.25x10^{7}$	$9.15x10^{4}$	$7.49x10^{4}$	$1.28x10^{2}$	$3.17x10^{2}$
	Std.Dev.	$6.45x10^{7}$	$3.48x10^{5}$	$3.58x10^{5}$	$3.63x10^{1}$	$9.87x10^{1}$
Chlorinated	Max.	$1.88x10^{7}$	$1.64x10^{3}$	$1.25x10^{3}$	$9.20x10^{1}$	$2.76x10^{2}$
Effluent	Min.	$1.01x10^{6}$	$3.72x10^{2}$	$2.00x10^{2}$	$4.00x10^{1}$	$1.18x10^{2}$
	Mean	$9.83x10^{6}$	$7.65x10^{2}$	$7.46x10^{2}$	$6.50x10^{1}$	$1.94x10^{2}$
	Median	$6.71x10^{6}$	$6.89x10^{2}$	$6.71x10^{2}$	$6.26x10^{1}$	$1.87x10^{2}$
	Std.Dev.	$6.26x10^{6}$	$4.14x10^{2}$	$3.45x10^{2}$	$1.72x10^{1}$	$5.11x10^{1}$
*Dry	Max.	$8.74x10^{8}$	$7.52x10^{8}$	$1.69x10^{7}$	$2.60x10^{5}$	$3.90x10^{4}$
Sludge	Min.	$6.74x10^{5}$	$9.52x10^{6}$	$7.07x10^{5}$	$3.00x10^{3}$	$2.80x10^{3}$
	Mean	$3.22x10^{8}$	$2.58x10^{8}$	$7.82x10^{6}$	$9.13x10^{4}$	$1.07x10^{4}$
	Median	$2.53x10^{7}$	$9.95x10^{6}$	$4.77x10^{5}$	$3.99x10^{3}$	$8.09x10^{2}$
	Std.Dev.	$2.30x10^{8}$	$1.96x10^{8}$	$4.95x10^{6}$	$6.69x10^{4}$	$1.12x10^{4}$

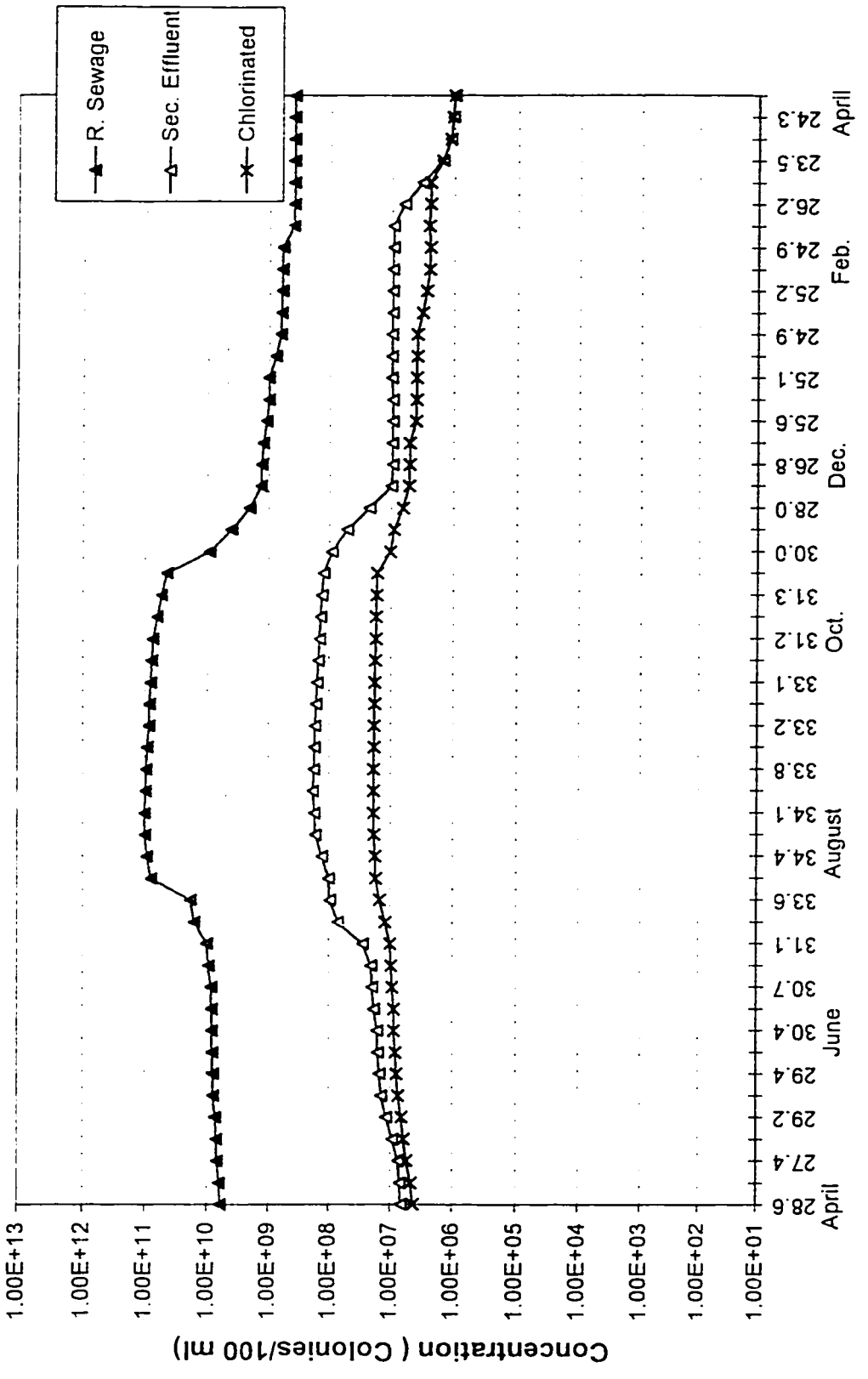
SPC = Standard Plate Count (colonies/100ml)
TC = total coliform (MPN/100ml)
FC = fecal coliform (MPN/100ml)
CP = coliphage (PFU/100ml)
Cl.Pr = *Cl. perfringens* (colonies/100ml)
*Dry sludge numbers are reported as per gram of sludge

5. 3 Seasonal Variations in the Microbial Population Density

The variation in the indicator microorganism population density with the variation of climatic season was monitored over a period of one year at the Al-Khobar sewage treatment plant. The data obtained during this study was analyzed logically. In addition to this, data was also analyzed statistically to reinforce the conclusions drawn on the basis of results obtained.

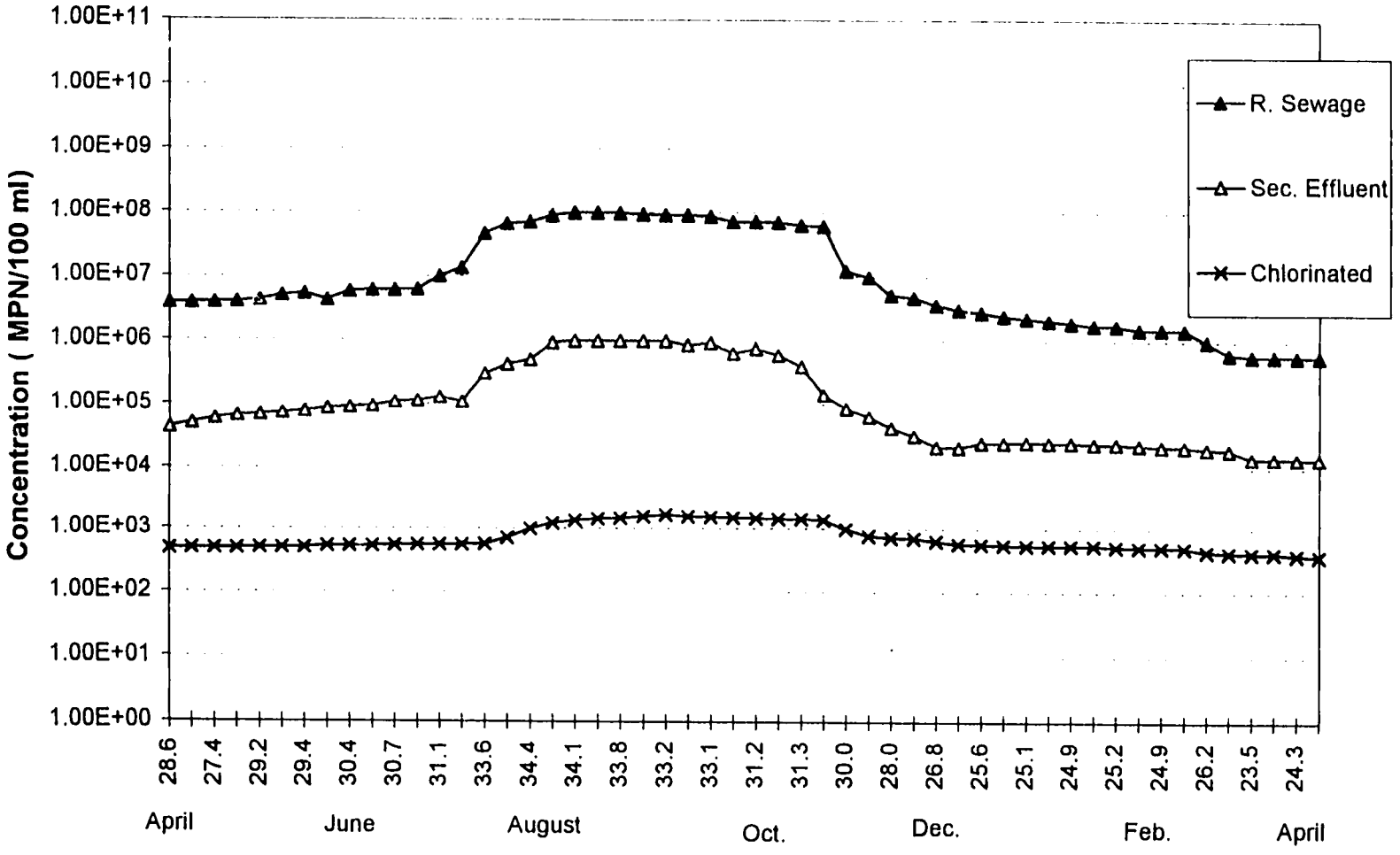
A clear pattern of increase in all microorganisms was observed during summer season, as can be seen in Fig 5.3. to Fig 5.7. The increase in the microbial population density with the temperature is due to the fact that the generation time of microorganisms generally decreases with an increase in temperature. Stokes (1968) reported that, at an increase of 5°C from 0°C the generation time of Psychrophilic Bacillus sp decreases from 23 hr to 8.5hr and this time reduced to 2.5 hr at about 20°C. Another supporting observation is one reported by Hawkes (1963) which states that the growth rates of microorganisms double with approximately every 10°C increase in temperature until the optimum temperature is reached.

Seasonal variation in the Standard Plate Count density at Al-Khobar wastewater treatment plant is shown in Fig 5.3. It is clear from the figure that the Standard Plate Count in raw sewage, secondary effluent, and chlorinated effluents is high during summer season and when temperature of wastewater



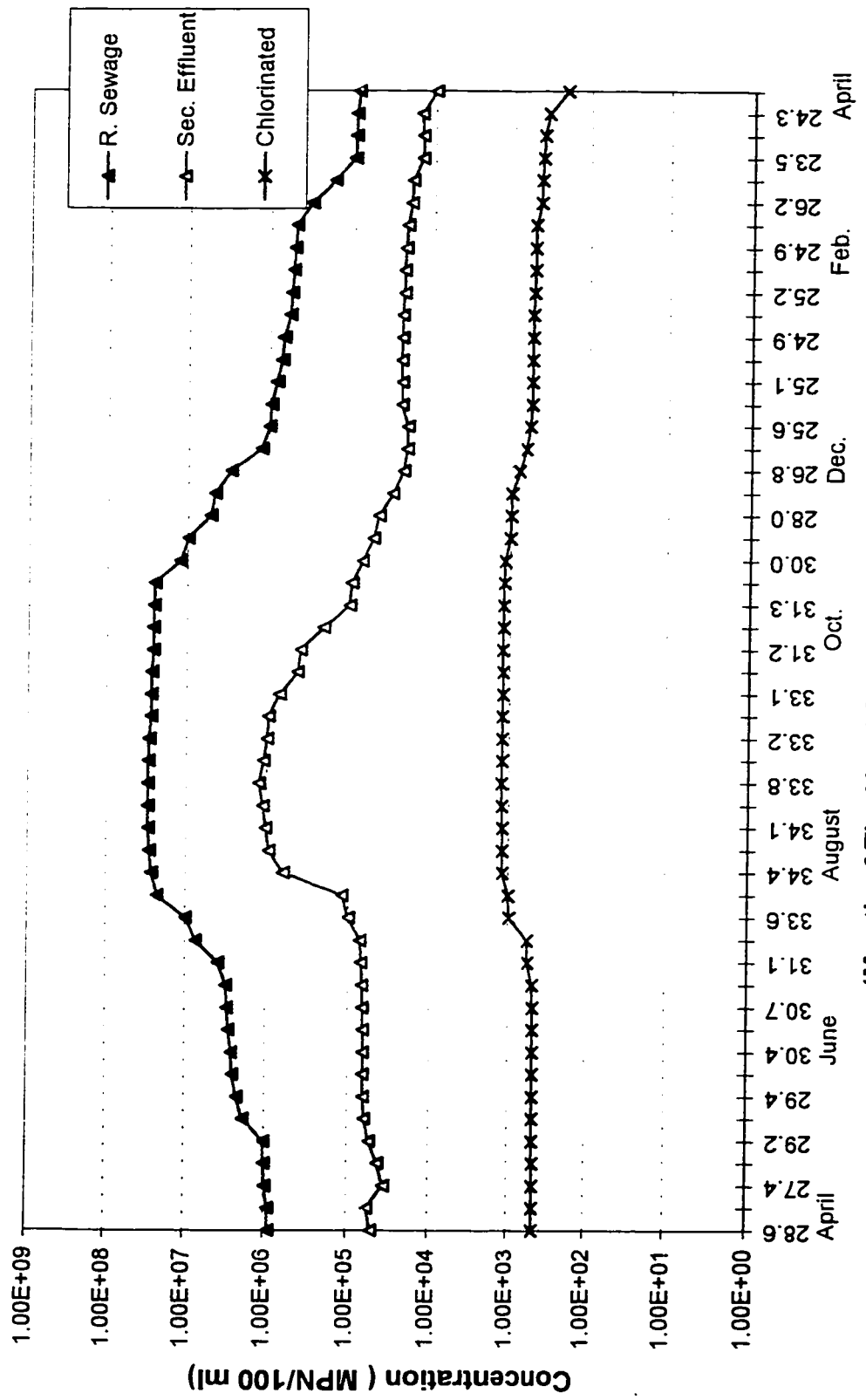
(Month of The Year/ Avg. Weekly Temperature)

Fig 5.3: Seasonal Variation of Standard Plate Count at Al-Khobar STP



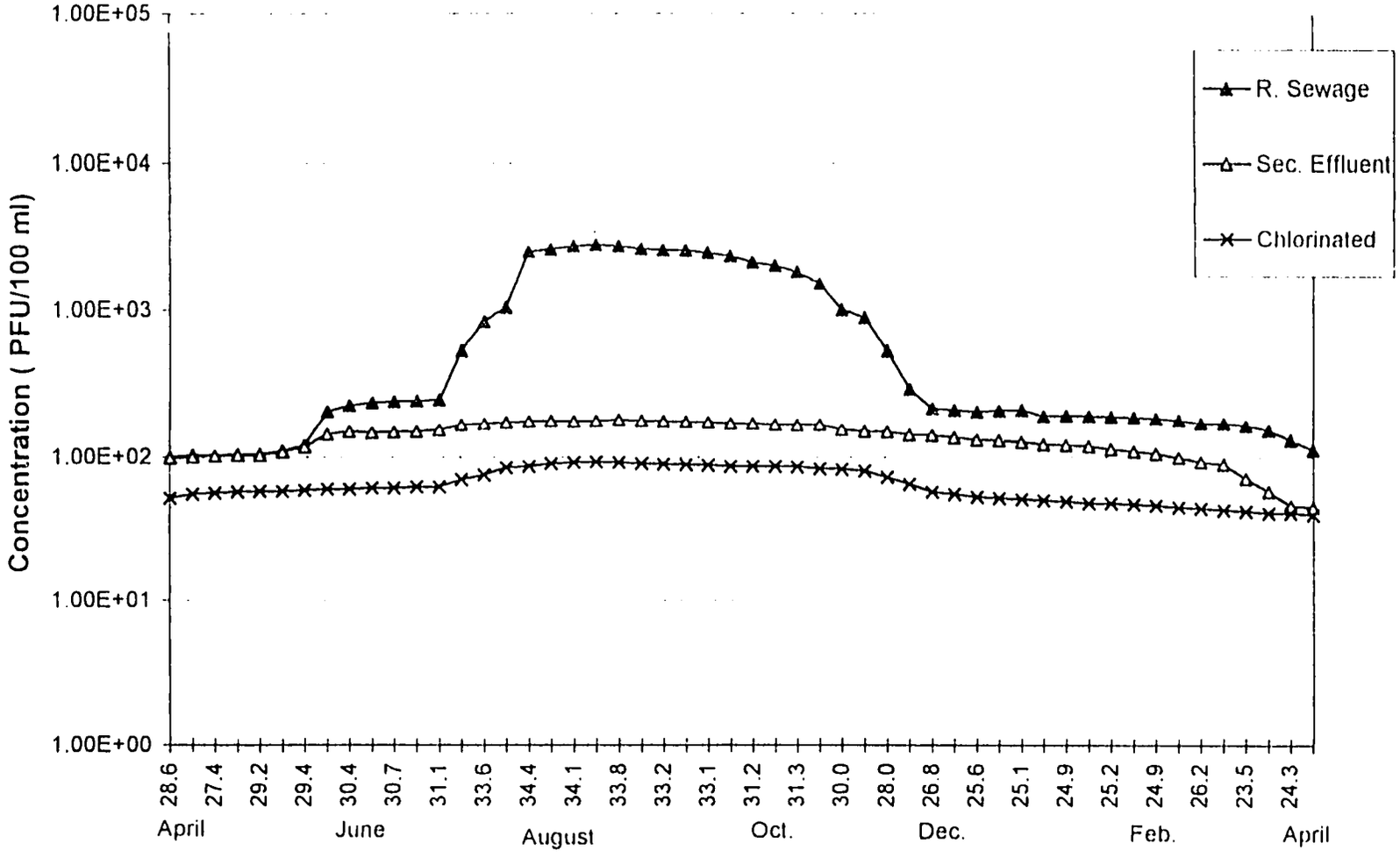
(Month of The Year/ Avg. Weekly Temperature)

Fig 5.4: Seasonal Variation of Total Coliform Population at Al-Khobar STP



(Month of The Year/ Avg. Weekly Temperature)

Fig 5.5:Seasonal Variation of Fecal Coliform Population at Al-Khobar STP



(Month of The Year/ Avg. Weekly Temperature)

Fig 5.6: Seasonal Variation of Coliphage Population at Al-Khobar STP

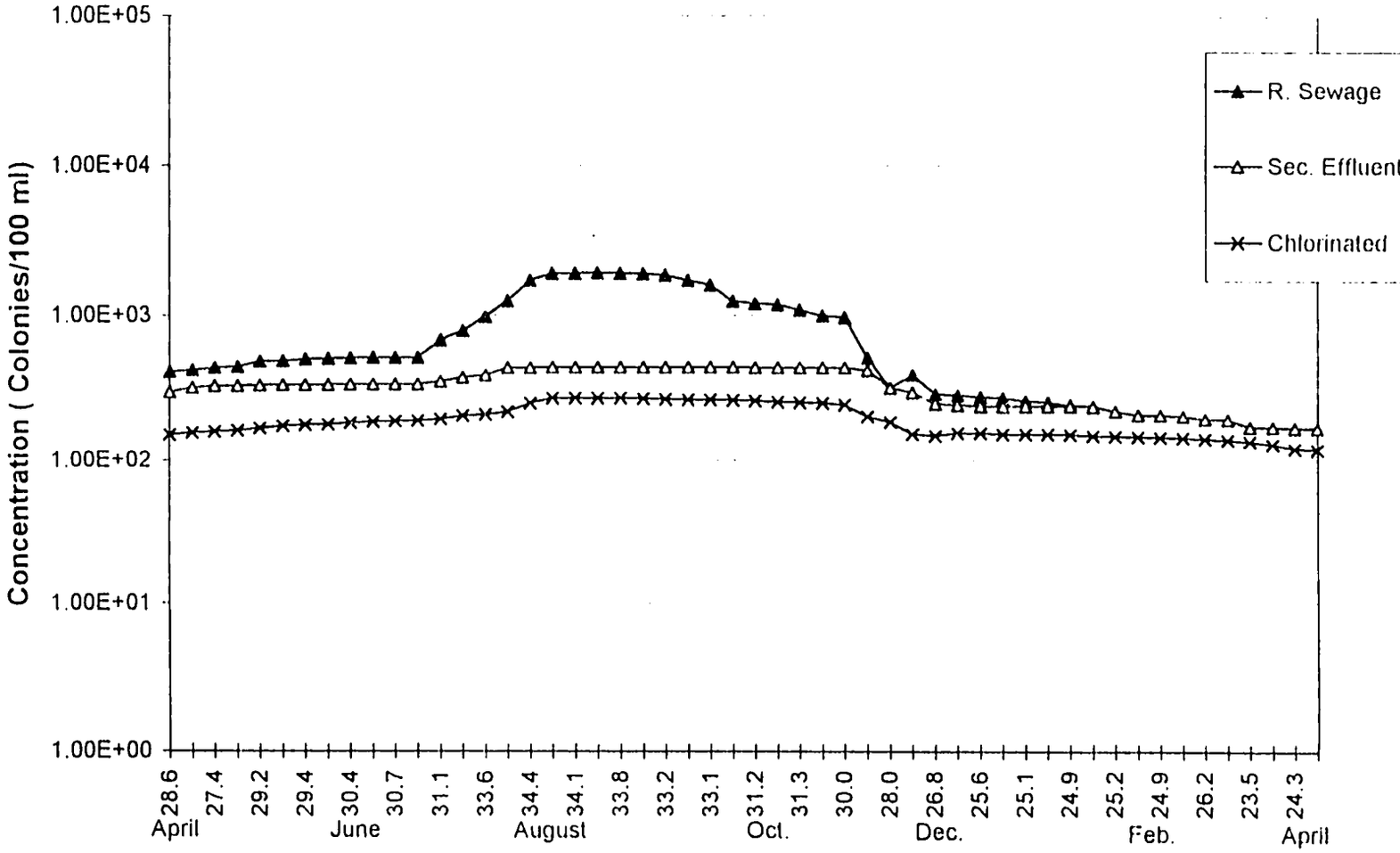


Fig 5.7: Seasonal Variation of *Cl. perfrengens* Population at Al-Khobar STP

increases to 34°C the Standard Plate Count density reaches its maximum observed value. With the onset of winter Standard Plate Count density starts decreasing and reaches up to its minimum observed value when temperature reaches about 24°C.

Total coliform is a well known and globally accepted indicator microorganism. If the behavior of this microorganism with the change in season is known it may be beneficial for the evaluation of any wastewater treatment system. Change in the total coliform population density with season is shown in Fig 5.4. A pattern similar to the Standard Plate Count can be can be observed from this figure. The trend of the total coliform population density shows that the effect of wastewater temperature is more in the raw sewage than it is in secondary effluent and least in the chlorinated effluent. This attribute may be due to the fact that wastewater spends 10 to 17 hr of detention time in aeration tanks and about 3.7 hr in secondary clarifiers, which may tend to moderate the temperature and consequently the change in microbial population density.

Similarly change in the population density of fecal coliform with the season is shown in Fig 5.5. Behavior of fecal coliform population density is similar to the total coliform and it is logical because fecal coliform is a sub-group of total coliform. Fig 5.5 shows that the least change in the population density of fecal coliform with the season occurs in the chlorinated effluent. One reason for this attribute may be defined by Van't Hoff-Arrhenius relationship "increase in the temperature of chlorinated water results in a more rapid kill" (MetCalf & Eddy,

1991). Earlier Sterritt and Lester (1988) stated that about 50% more free available chlorine would be required for effective disinfection at < 5°C compared with 10°C. It means that during summer effect of chlorine on microbial population was more than in winter and as a results less increase in the microbial population was observed during summer in the chlorinated effluent.

Variation of the viral indicator, coliphage, population density with the season is shown in Fig 5.6. Coliphage density during summer season was higher than those in winter season in the raw sewage and the difference decreases in successive effluents. This attribute is also reported by Rao et al. (1974) during a quantitative evaluation of virus removal in an activated sludge treatment plant. The authors reported a higher count of viruses during summer than in winter, but the difference was reduced in the final effluents.

Change in the population density of *Cl. perfringens* is presented in Fig 5.7. Population density is higher during summer than it is in winter, but this increase in its population is insignificant compared to other indicator microorganisms. Similarly difference in population density decreases in successive effluents. *Cl. perfringens* is highly resistant against high temperature and form a protective spore during adverse environmental conditions. During this period *Cl. perfringens* do not multiply but remain inactive (Michel, 1974). Hence, population density of *Cl. perfringens* did not increase significantly during summer in wastewater.

In order tc compare the effect of summer and winter season on microbial population density, data obtained during summer and winter have been

analyzed. Average population density of microorganisms during summer is higher than during winter. It was observed that Standard Plate Counts have more than two log order high population in summer, total and fecal coliforms both have more than one log order high population in summer as compared to in winter. Coliphage and *Cl. perfringens* have less than one log higher population density in summer as compared to it is in winter. Values of range, mean, median, and standard deviation obtained during summer and winter is shown in Table 5.2 and Table 5.3. A comparison of microbial population density during summer and winter is presented in Fig 5.8. Comparison clearly shows that there is a significant difference in the population densities of Standard Plate Count, total coliform, fecal coliform, and coliphage during summer and winter but difference in the population density of *Cl. perfringens* is insignificant. This may be due to the reason *Cl. perfringens* do not multiply and is incapable of proliferating because of its extremely high nutrient requirement (Boyd et al., 1948; Fuchs and Bond, 1957).

Table 5.2: Microbial Population Density in Different Effluents and Dry Sludge (Summer)

Sample		SPC	TC	FC	CP	Cl.Pr
Raw	Max.	$1.01x10^{11}$	$9.80x10^{7}$	$2.93x10^{7}$	$2.80x10^{3}$	$1.93x10^{3}$
Sewage	Min.	$6.00x10^{9}$	$3.80x10^{6}$	$8.81x10^{5}$	$1.00x10^{2}$	$4.21x10^{2}$
	Mean	$3.01x10^{11}$	$3.11x10^{7}$	$9.81x10^{7}$	$3.73x10^{2}$	$2.58x10^{2}$
	Median	$7.23x10^{10}$	$1.82x10^{7}$	$1.36x10^{7}$	$3.10x10^{2}$	$2.10x10^{2}$
	Std.Dev.	$3.84x10^{10}$	$3.85x10^{7}$	$1.24x10^{7}$	$1.12x10^{3}$	$5.78x10^{2}$
Secondary	Max.	$1.82x10^{8}$	$9.93x10^{5}$	$1.23x10^{6}$	$1.80x10^{2}$	$4.53x10^{2}$
Effluent	Min.	$6.57x10^{6}$	$4.34x10^{4}$	$3.59x10^{4}$	$9.80x10^{1}$	$3.04x10^{2}$
	Mean	$7.76x10^{7}$	$7.21x10^{6}$	$1.06x10^{7}$	$1.87x10^{2}$	$1.62x10^{2}$
	Median	$1.75x10^{7}$	$1.39x10^{6}$	$8.87x10^{5}$	$1.82x10^{2}$	$9.97x10^{1}$
	Std.Dev.	$6.85x10^{7}$	$3.81x10^{5}$	$4.19x10^{5}$	$2.86x10^{1}$	$5.49x10^{1}$
Chlorinated	Max.	$1.88x10^{7}$	$1.64x10^{3}$	$1.25x10^{3}$	$9.20x10^{1}$	$2.76x10^{2}$
Effluent	Min.	$4.17x10^{6}$	$4.73x10^{2}$	$4.59x10^{2}$	$5.20x10^{1}$	$1.52x10^{2}$
	Mean	$2.08x10^{3}$	$1.81x10^{3}$	$9.85x10^{2}$	$9.46x10^{1}$	$1.38x10^{2}$
	Median	$1.71x10^{3}$	$1.36x10^{3}$	$8.58x10^{2}$	$6.97x10^{1}$	$9.76x10^{1}$
	Std.Dev.	$5.27x10^{6}$	$4.68x10^{2}$	$3.68x10^{2}$	$1.45x10^{1}$	$4.48x10^{1}$
*Dry	Max.	$7.34x10^{8}$	$4.52x10^{8}$	$9.66x10^{6}$	$8.47x10^{4}$	$3.89x10^{4}$
Sludge	Min.	$6.74x10^{5}$	$9.52x10^{6}$	$7.07x10^{5}$	$3.00x10^{3}$	$2.82x10^{3}$
	Mean	$3.22x10^{8}$	$1.23x10^{8}$	$5.27x10^{6}$	$4.13x10^{4}$	$1.37x10^{4}$
	Median	$1.78x10^{8}$	$2.35x10^{8}$	$1.01x10^{6}$	$8.75x10^{3}$	$3.17x10^{3}$
	Std.Dev.	$2.23x10^{8}$	$1.39x10^{8}$	$3.61x10^{6}$	$3.15x10^{4}$	$1.32x10^{4}$

SPC = Standard Plate Count (colonies/100ml)
TC = total coliform (MPN/100ml)
FC = fecal coliform (MPN/100ml)
CP = coliphage (PFU/100ml)
Cl.Pr = *Cl. perfringens* (colonies/100ml)
*Dry sludge numbers are reported as per gram of sludge

Table 5.3: Microbial Population Density in Different Effluents and Dry Sludge (Winter)

Sample		SPC	TC	FC	CP	Cl.Pr
Raw	Max.	9.07×10^{9}	1.24×10^{7}	1.22×10^{7}	9.98×10^{2}	9.69×10^{2}
Sewage	Min.	3.86×10^{8}	5.85×10^{5}	9.05×10^{4}	1.12×10^{2}	1.70×10^{2}
	Mean	7.02×10^{8}	1.76×10^{6}	2.73×10^{6}	1.52×10^{2}	1.92×10^{2}
	Median	2.10×10^{8}	4.37×10^{5}	6.54×10^{4}	9.52×10^{1}	1.33×10^{2}
	Std.Dev.	1.92×10^{9}	3.00×10^{6}	3.25×10^{6}	2.32×10^{2}	1.73×10^{2}
Secondary	Max.	8.94×10^{7}	8.49×10^{4}	7.34×10^{4}	1.53×10^{2}	4.45×10^{2}
Effluent	Min.	1.02×10^{6}	1.42×10^{4}	1.01×10^{4}	4.50×10^{1}	1.70×10^{2}
	Mean	8.47×10^{6}	1.66×10^{4}	1.63×10^{4}	7.95×10^{1}	1.53×10^{2}
	Median	1.44×10^{6}	1.20×10^{4}	1.18×10^{4}	6.33×10^{1}	1.53×10^{2}
	Std.Dev.	1.97×10^{7}	1.67×10^{4}	1.43×10^{4}	3.26×10^{1}	7.36×10^{1}
Chlorinated	Max.	1.03×10^{7}	9.82×10^{2}	1.18×10^{3}	8.20×10^{1}	2.46×10^{2}
Effluent	Min.	1.01×10^{6}	3.72×10^{2}	2.00×10^{2}	4.00×10^{1}	1.18×10^{2}
	Mean	2.20×10^{6}	3.97×10^{2}	3.78×10^{2}	3.62×10^{1}	1.23×10^{2}
	Median	1.14×10^{6}	3.58×10^{2}	2.90×10^{2}	3.60×10^{1}	1.18×10^{2}
	Std.Dev.	2.35×10^{6}	1.43×10^{2}	2.46×10^{2}	1.21×10^{1}	2.76×10^{1}
*Dry	Max.	8.74×10^{8}	7.52×10^{8}	1.69×10^{7}	2.64×10^{5}	2.90×10^{4}
Sludge	Min.	2.85×10^{6}	2.39×10^{7}	1.68×10^{6}	6.23×10^{3}	2.83×10^{3}
	Mean	6.78×10^{8}	1.99×10^{8}	5.63×10^{6}	7.90×10^{4}	5.50×10^{3}
	Median	2.39×10^{6}	4.41×10^{7}	2.19×10^{6}	1.71×10^{4}	2.62×10^{3}
	Std.Dev.	2.44×10^{8}	2.20×10^{8}	5.52×10^{6}	7.82×10^{4}	7.23×10^{3}

SPC = Standard Plate Count (colonies/100ml)
TC = total coliform (MPN/100ml)
FC = fecal coliform (MPN/100ml)
CP = coliphage (PFU/100ml)
Cl.Pr = *Cl. perfringens* (colonies/100ml)
*Dry sludge numbers are reported as per gram of sludge

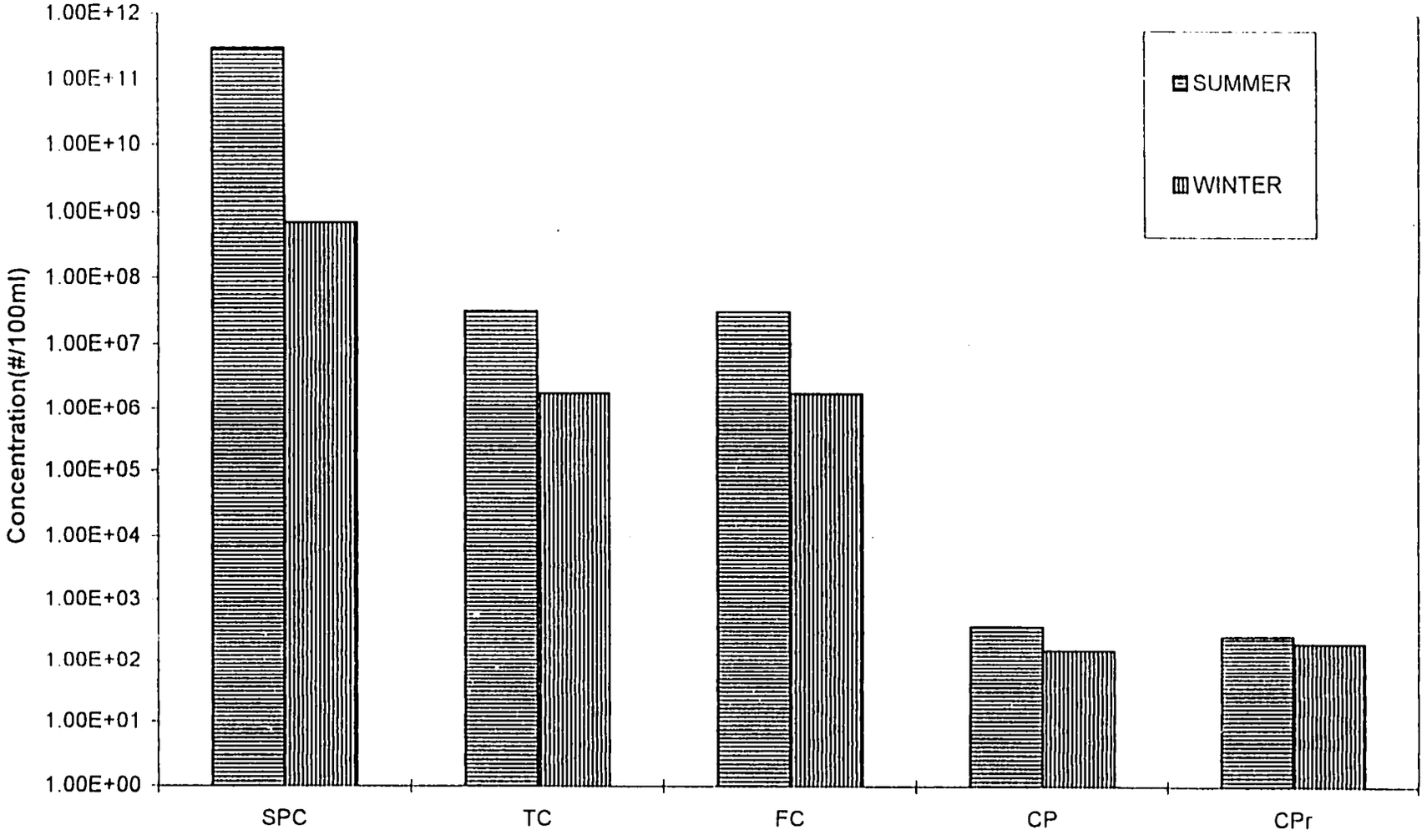


Fig. 5.8: Comparision of Indicator Microorganism Population in Raw Sewage of Al-Khobar STP (Summer and Winter)

In order to verify the pattern of the data obtained, statistical tools have been used in this study. The t-test analysis on microbial population densities has been performed to see whether summer and winter values were significantly different or not. Analysis was carried out at a confidence level of 95 percent. This implies that, there is a confidence that 95% of time there is a significant difference in the means of summer and winter data and only 5% probability that their means are equal. In this study, all t-test results are stated at 95% confidence level, and presented in Table 5.4.

The results of t-test analysis reinforce the logical analysis made during this study. The microbial population densities were significantly different during summer and winter. Microbial population in raw sewage, secondary effluent, and chlorinated effluents followed the same pattern. Further analysis showed that the summer values are always higher than winter values. These findings were in agreement with the study of many researchers (Rao, et al., 1974; Gaudy & Gaudy, 1980; Farooq et al., 1997).

Table 5.4: Statistical Comparison of Summer and Winter Microbial Population Density using t-test @ 95% Confidence Interval

		SUMMER DATA			WINTER DATA							
Sample	Microb	Sample Size (n_1)	Mean (Y_1)	Variance S_1^2	Sample Size (n_2)	Mean (Y_2)	Variance S_2^2	t_{cal}	t_{tab}	D.F	Null Hypoth	Inference
Raw	SPC	30	4.49×10^{10}	1.48×10^{21}	22	1.30×10^{9}	3.67×10^{18}	6.2018	2.045	29	Reject Ho	summer > winter
Sewage	TC	30	4.44×10^{7}	1.48×10^{15}	22	2.82×10^{6}	8.98×10^{12}	5.8996	2.045	29	Reject Ho	summer > winter
	FC	30	1.49×10^{7}	1.54×10^{14}	22	1.99×10^{6}	1.05×10^{13}	5.4616	2.0336	34	Reject Ho	summer > winter
	CP	30	1.49×10^{7}	1.26×10^{6}	22	2.71×10^{2}	5.3	4.7474	2.0378	32	Reject Ho	summer > winter
	C.Pr	30	1.06×10^{3}	3.34×10^{5}	22	2.86×10^{2}	3.00×10^{4}	6.9115	2.0294	36	Reject Ho	summer > winter
Sec.	SPC	30	8.67×10^{7}	4.69×10^{15}	22	1.37×10^{7}	3.89×10^{14}	5.4401	2.0315	35	Reject Ho	summer > winter
Effluent	TC	30	4.19×10^{5}	1.45×10^{11}	22	2.74×10^{4}	2.79×10^{8}	5.6235	2.045	29	Reject Ho	summer > winter
	FC	30	3.65×10^{5}	1.75×10^{11}	22	2.65×10^{4}	2.04×10^{8}	4.4231	2.045	29	Reject Ho	summer > winter
	CP	30	1.51×10^{2}	8.18×10^{2}	22	1.11×10^{2}	1.06×10^{3}	4.7059	2.0189	42	Reject Ho	summer > winter
	C.Pr	30	3.97×10^{2}	3.01×10^{3}	22	2.44×10^{2}	5.42×10^{3}	8.1902	2.0273	37	Reject Ho	summer > winter

Table. 5.4: Continued

		SUMMER DATA			WINTER DATA							
Sample	Microb	Sample Size (n_1)	Mean (Y_1)	Variance S_1^2	Sample Size (n_2)	Mean (Y_2)	Variance S_2^2	t_{cal}	t_{tab}	D.F	Null Hypoth	Inference
Chlori-nated Effluent	SPC	30	1.29×10^{7}	2.77×10^{13}	22	3.74×10^{6}	5.53×10^{12}	8.5059	2.0178	43	Reject Ho	summer > winter
	TC	30	9.43×10^{2}	2.19×10^{5}	22	5.49×10^{2}	2.04×10^{4}	4.3453	2.0294	36	Reject Ho	summer > winter
	FC	30	8.58×10^{2}	1.35×10^{5}	22	5.94×10^{2}	6.07×10^{4}	3.0938	2.0105	50	Reject Ho	summer > winter
	CP	30	7.40×10^{1}	2.11×10^{2}	22	5.23×10^{1}	1.46×10^{2}	5.8629	2.0115	49	Reject Ho	summer > winter
	C.Pr	30	2.22×10^{2}	2.01×10^{3}	22	1.54×10^{2}	7.63×10^{2}	6.7661	2.0115	49	Reject Ho	summer > winter

5. 4 Variation of Indicator Microorganism Population Density in Different effluents of the Plant

A prominent difference in microbial population density is observed in different effluents of the Al-Khobar STP. Average density of total coliform and fecal coliform in the raw sewage is found as $3.48x10^7$ MPN/100 ml and $1.61x10^7$ MPN/100ml. Similarly average count of Standard Plate Counts, *Cl. perfringens* and Coliphage were $4.71x10^{10}$ colonies/100 ml, $7.51x10^2$ colonies/100 ml and $8.26x10^2$ PFU/100 ml respectively. A decrease in the microbial population density was observed in the secondary effluent of the treatment plant. This is mainly due to the settling of the microorganisms in the secondary clarifier. Average reduction of total coliform and fecal coliform in the secondary effluent observed to be 99.22% and 98.78% respectively. Similarly average reduction in Standard Plate Counts, coliphage and *Cl. perfringens* were 99.85%, 83.62% and 55.5% respectively.

In a study carried out by Rao et al. (1974) on a conventional activated sludge treatment plant a virus removal of about 99% has been reported. This higher value may be due to the fact that they used 24-hour composite sampling technique for sample collections. In Standard Methods (1995) grab sampling techniques were recommended for analysis of biological parameters because, composite samples are not representative of actual population of microorganisms at the time of enumeration. A composite 24-hour sampling may

results in furthe reduction of microorganisms before laboratory analysis and results in wrong prediction of their removal efficiency.

Similarly, Omura et al. (1989) carried out a study on removal efficiencies of indicator microorganisms in different sludge treatment plants. Coliform and entrococcus bacteria were used as bacterial indicators and coliphage acted as an viral indicator. Authors reported a removal of bacteria up to 97% and viruses up to 96.6% in secondary treated effluents. They justified higher removal of coliphage as due to the higher adsorption ability of conventional viruses on MLSS of activated sludge treatment processes. In our study less removal of coliphage could be due to the absence of primary settling tank in carrousel type activated sludge treatment plant. Gerba (1981), reported that, an average 50% removal of viruses could be obtained with an average settling time of 2 hours. Rao et al. (1974) reported similar results in his virus removal study in a conventional activated sludge treatment plant.

Cl. perfringens has least removal after secondary treatment as compared with other indicator microorganisms studied. Similar results have been reported by Hirata et al. (1991) in the evaluation study of wastewater and sludge treatment system. They used ten microorganisms for their study E.coli, aeruginosa, and *Cl. perfringens* were among the microorganisms studied. They reported that *Cl. perfringens* was the least reduced in almost all unit processes, compared with the other indicator microorganisms studied. Little information about *Cl. perfringens* in secondary treated effluents is available in the literature.

Researchers generally are interested in the high resistance of *Cl. perfringens* against chlorination rather than its removal in the secondary treatment. In this regard the current study has an academic value for further research in this area.

As chlorination is carried out as a disinfection process in the chlorination unit, the microbial population density decreases tremendously after chlorination. Residual chlorine monitored during this study ranges from 0 to 0.5 mg/l. Average population density of total coliform and fecal coliform in the chlorinated effluent reaches to 7.65 $x10^2$ MPN/100 ml and 7.46 $x10^2$ MPN/100 ml respectively. Similarly average values of Standard Plate Counts, and coliphage were 9.83 $x10^6$ colonies/100 ml, and 65.0 PFU/100 ml. Only *Cl. perfringens* shows a minimum decrease in the population density after chlorination with an average of 1.94 $x10^2$ colonies/100 ml. These values correspond to a removal of 99.62%, 86.27%, 99.72%, 51.96%, and 42.1% for Standard Plate Counts, total coliform, fecal coliform coliphage and *Cl. perfringens* respectively.

A comparison of percent removal of different indicator microorganisms with some values reported in literature is presented in Table 5.5. Percent removal of all indicator microorganisms studied are comparable and some variations in percent removal may be due to some inherent differences in actual field study and pilot plant studies. Similarly use of liquid chlorine mixture instead of chlorine gas in the study by Imran (1997) could be one of the reason for these differences.

Table 5.5: Comparison of Percent Removal of Indicator Microorganisms During Different Treatments with Some Reported Values.

Parameter	Secondary Treatment		Chlorination	
	Al-Khobar	Literature	Al-Khobar	Literature
SPC	99.85	--	86.27	87.3[d]
TC	99.22	76 - 99[a]	99.72	99.99[e]
FC	98.78	96 - 99.4[b]	99.62	84.9 [d]
CP	83.62	90 - 99.1[c]	51.96	49.1 [d]
C.Pr	55.50	--	42.07	44.5 [d]

[a] After Lance, (1983).

[b] After Clark et al., (1961).

[c] After Rao et al., (1979).

[d] After Imran, (1997).

[e] After Omura et al., (1989).

It is clear from Table 5.5 that *Cl. perfringens* is the only microorganism that is least reduced after chlorination. This behavior of *Cl. perfringens* is mainly due to the property of spore formation of this anaerobic bacteria. Mitchel (1974) stated that the spore-forming property increases the survival rate of microorganisms because by this protective spore they can resist adverse environmental conditions. *Cl. perfringens* does not multiply and is incapable of proliferating even in nutrient-rich sewage, because of its extremely high nutrient requirements (Boyd et al., 1948; Fuchs and Bond, 1957). Hirata et al. (1991), in a study for the evaluation of the effect of wastewater and sludge treatment system using *Cl. perfringens* as an indicator reported that, *Cl. perfringens* has high resistance against chlorination. They also reported that the resistance of *Cl. perfringens* in sewage was 3 to 5 times higher than that of pure culture.

A study was carried out by Tyrrell et al. (1995) for a comparison of inactivation of bacterial and viral indicators using chlorine and ozone. They used fecal coliform, entrococci, *Cl. perfringens*, male-specific bacteriophage and somatic coliphage. The authors reported that *Cl. perfringens* is relatively insensitive to inactivation by either disinfectant. They stated that the spore-forming characteristic of *Cl. perfringens* is responsible for their stability after prolonged contact periods. This behavior is also reported by Wilson and Blair, (1925).

A comparison of average population density of all studied indicator microorganisms in different effluents of the Al- Khobar STP is presented in Fig 5.9. It is clear from the Fig 5.9 that the reduction of *Cl. perfringens* is insignificant and maximum reduction of total coliform and fecal coliform is achieved after chlorination. The removal of total coliform and fecal coliform is similar which may be due to the fact that fecal coliform is a sub-group of total coliform and exhibits a similar behavior. The reason for this attribute is depicted by Liu et al. (1971). They reported that this group is less resistant against chlorination than the least chlorine resistant virus (reovirus1), which required only 2.7 minutes contact time with 0.5mg/l free chlorine for 99.99% kill. Standard Plate Count comes next in the reduction observed. This behavior could be due to the fact that Standard Plate Count comprises of all aerobic and facultative aerobic hetrotropic bacteria (Standard Methods, 1995). It may be possible that among the large number unknown bacteria there is a significant number of sensitive or less resistant microorganisms present and results in higher reduction after chlorination. Similar reasons were given by Liu et al. (1971).

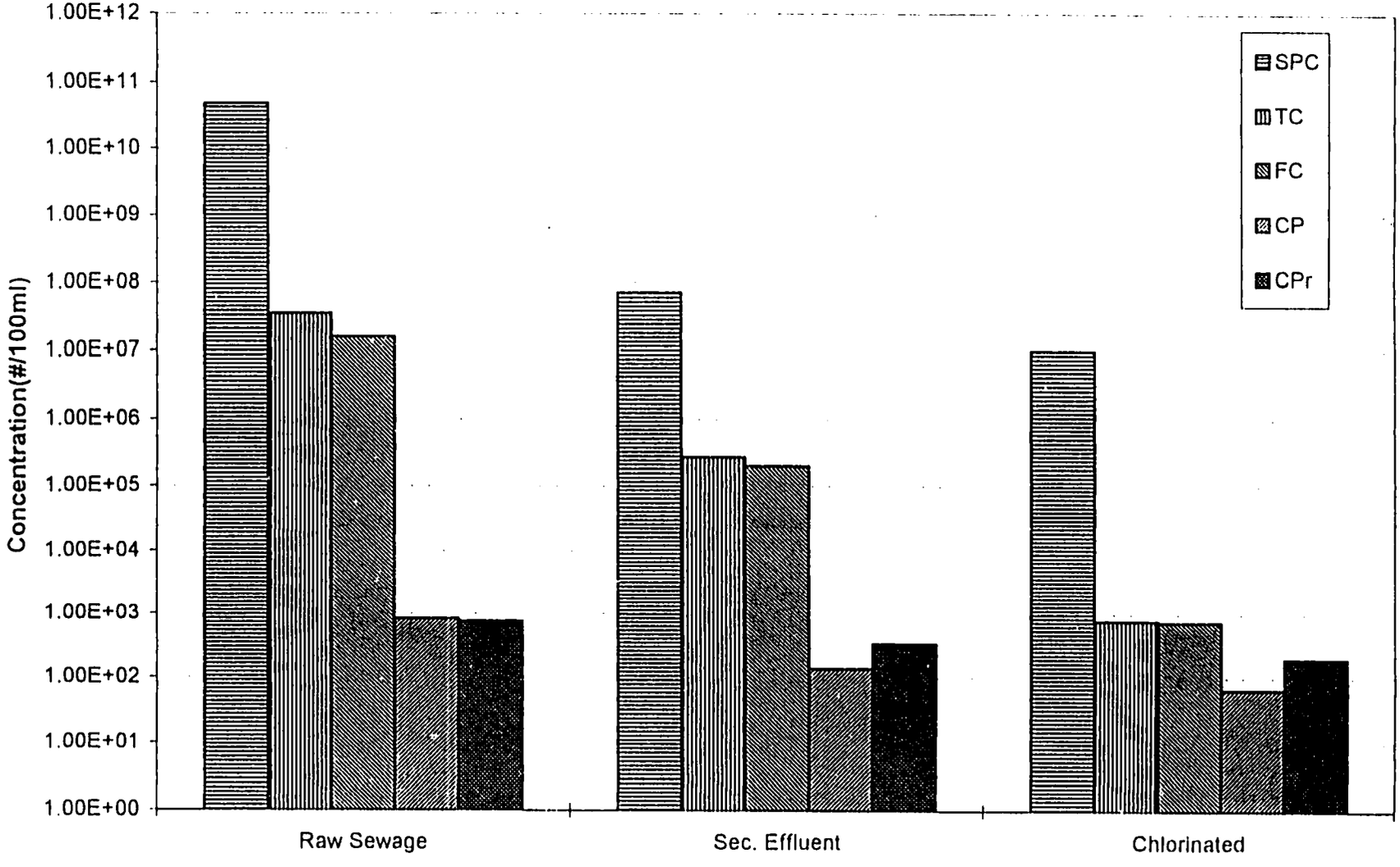


Fig. 5.9: Population Density of Indicator M.O in Different Effluent of Al-Khobar STP (Annual)

5. 5 Regression Modeling of Indicator Microorganism Population Densities in Different Effluents of Al-Khobar STP.

One of the objective of this research study was to find out the effect of seasonal variations on microorganism population densities at different level of treatment. In order to achieve this goal microbial population densities at corresponding temperature and flowrate were evaluated and statistical analysis were performed to reinforce our results. One of the best way to achieve this goal is the use of regression analysis (described in sec 4.6.2) for the modeling.

Regression analysis was applied for each parameter in the different effluents of the treatment plant. Regression equations were obtained, in order to correlate the microbial population densities with water temperature and flow rate. The values of F-calculated, P-value, R^2, R^2-adjusted, and Standard error of regression analysis are presented in Table 5.6.

In regression analysis of Standard Plate Count population density present in raw sewage, R^2 statistic indicates that Model-1 explains 84.0% of the variability of independent variables. The R^2- adjusted statistic which is more suitable for comparing models with different numbers of independent variables, is 82.6%. The standard error of the estimate shows the standard deviation of the residuals. Since P-value in each case is less than 0.05, there is significant relationship between the variables at more than 95% confidence interval.

Table 5.6: Statistic of Regression Analysis for Various Indicators Present in Different Effluents of Al-Khobar STP.

Sample	Parameter	F-cal	P-value	R^2	R^2 adjusted	Std. Error
Raw	SPC	61.57	< 0.001	0.840	0.826	$1.51x10^{+10}$
Sewage	TC	50.58	< 0.001	0.812	0.795	$1.61x10^{+7}$
	FC	36.94	< 0.001	0.759	0.738	$5.91x10^{+6}$
	CP	36.29	< 0.001	0.755	0.735	$5.12x10^{+2}$
	C.Pr	71.23	< 0.001	0.817	0.805	$2.61x10^{+2}$
Secondary	SPC	46.03	< 0.001	0.797	0.780	$3.03x10^{+7}$
Effluent	TC	43.36	< 0.001	0.787	0.769	$1.67x10^{+5}$
	FC	30.12	< 0.001	0.719	0.695	$1.97x10^{+5}$
	CP	39.33	< 0.001	0.770	0.750	18.13
	C.Pr	90.57	< 0.001	0.850	0.840	39.41
Chlorinated	SPC	98.42	< 0.001	0.893	0.884	$2.13x10^{+6}$
Effluent	TC	15.29	< 0.001	0.566	0.529	$2.84x10^{+2}$
	FC	16.84	< 0.001	0.589	0.554	$2.31x10^{+2}$
	CP	45.71	< 0.001	0.796	0.778	8.12
	C.Pr	54.85	< 0.001	0.824	0.809	22.36

The F-cal value is 61.57 which is more than the $F_{0.05,4,47} = 8.37$, given in standardized tables (Montgomery, 1981). The "0.05" is the confidence interval and "4" and "47" are the degree of freedom for the regression and error, respectively. The F-cal value of 61.57 corresponds to 99.9% of confidence interval. Almost all regression equations have good fit and the coefficient of determination (R^2) ranges from 0.566 (for total coliform in chlorinated effluent) to 0.893 (for Standard Plate Counts in chlorinated effluent).

There are, a number of factors involved in the fluctuations of the results but these equations are statistically sound to predict the results under some confidence bend. In this analysis confidence interval is automatically calculated by the program and defined by (1-P)-value. P-value presented in Table 5.6, showing confidence interval more than 99.99% in all cases. During the formation of regression equations few points were given considerable priority

1. Form the equation with least number of regressors
2. Avoid going for higher powers if found unnecessary

Finally a most general model for our case was developed.

$$Y = \alpha_o + \alpha_1 T + \alpha_2 T^2 + \alpha_3 T^{3/2} + \alpha_4 (FxT) + \alpha_5 T\sqrt{F} + \alpha_6 \ln T + \alpha_7 e^{\sqrt{T}} \qquad (5.1)$$

Where α_o, α_1, α_2,.....,α_7 are constants obtained from regression analysis and their values are given in Table 5.7.

Table 5.7: Regression Models For Various Indicator Microorganisms in Different Effluents of Al-Khobar STP.

Sample	Parameter	Model No.	Equation
Raw	SPC	1	$4.81\times10^{11} - 6.53\times10^{9}\,T + 2.56\times10^{5}\,FxT - 1.56\times10^{8}\,T\sqrt{F} + 1.85\times10^{9}\,e^{\sqrt{T}}$
Sewage	TC	2	$4.31\times10^{8} + 7.09\times10^{6}\,T + 3.58\times10^{2}\,FxT - 2.22\times10^{5}\,T\sqrt{F} + 1.69\times10^{5}\,e^{\sqrt{T}}$
	FC	3	$1.04\times10^{8} + 2.27\times10^{6}\,T + 98.74\,FxT - 5.99\times10^{4}\,T\sqrt{F} + 4.47\times10^{5}\,e^{\sqrt{T}}$
	CP	4	$1.25\times10^{4} + 3.16\times10^{2}\,T + 1.15\times10^{-2}\,FxT - 7.09\,T\sqrt{F} + 47.49\,e^{\sqrt{T}}$
	C.Pr	5	$-3.56\times10^{3} + 1.58\times10^{3}\,T + 1.44\times10^{-2}\,FxT - 9.09\,T\sqrt{F}$
Secondary	SPC	6	$6.04\times10^{8} + 2.86\times10^{7}\,T + 7.00\times10^{2}\,FxT - 4.37\times10^{5}\,T\sqrt{F} + 2.62\times10^{6}\,e^{\sqrt{T}}$
Effluent	TC	7	$4.17\times10^{6} + 2.89\times10^{5}\,T + 5.62\,FxT - 3.53\times10^{3}\,T\sqrt{F} + 1.64\times10^{4}\,e^{\sqrt{T}}$
	FC	8	$4.69\times10^{6} - 6.95\times10^{5}\,T + 9.98\,FxT - 6.28\times10^{3}\,T\sqrt{F} + 1.73\times10^{4}\,e^{\sqrt{T}}$
	CP	9	$-2.06\times10^{4} - 4.88\times10^{2}\,T + 2.16\times10^{-5}\,FxT + 9.88\times10^{3}\,\ln T + 7.32\,e^{\sqrt{T}}$
	C.Pr	10	$-4.31\times10^{2} + 98.23\,T + 6.90\times10^{-4}\,FxT - 0.45\,T\sqrt{F}$
Chlorinated	SPC	11	$2.29\times10^{6} + 2.04\times10^{6}\,T + 28.38\,FxT - 1.74\times10^{4}\,T\sqrt{F} + 1.08\times10^{5}\,e^{\sqrt{T}}$
Effluent	TC	12	$1.80\times10^{3} + 4.66\times10^{2}\,T + 6.12\times10^{-3}\,FxT - 3.74\,T\sqrt{F} + 8.67\,e^{\sqrt{T}}$
	FC	13	$-3.54\times10^{4} + 3.19\times10^{3}\,T - 1.05\times10^{2}\,T^{2} + 8.32\times10^{-2}\,T\sqrt{F} + 1.44\times10^{2}\,e^{\sqrt{T}}$
	CP	14	$-3.63\times10^{2} + 77.76\,T - 2.46\,T^{2} + 1.13\times10^{-3}\,T\sqrt{F} + 3.34\,e^{\sqrt{T}}$
	C.Pr	15	$-7.50\times10^{4} + 2.09\times10^{4}\,T + 5.53\,T^{2} - 6.17\times10^{3}\,T^{3/2} + 2.23\times10^{-3}\,T\sqrt{F} - 1.58\times10^{2}\,e^{\sqrt{T}}$

As it is clear from the Table 5.7. Regression equations for Standard Plate Counts, total coliform, fecal coliform and coliphage in raw sewage, secondary effluent, and chlorinated effluent are similar. Each equation has only four regressors and same form of the independent variables (temperature and flow rate). Only *Cl. perfringens* has relatively different equations consist of three regressors in raw sewage and secondary effluent and five in chlorinated effluent. This may be due to the different behavior of this spore-forming anaerobic bacteria. Another factor is its initial low population density in the influent and high resistant against temperature and disinfection.

5. 6 Verification of Regression Models

Values of the microbial population densities obtained experimentally were compared with the computed population densities (from regression models) for all studied indicator microorganisms. A typical comparison of computed and experimental values as shown in Fig 5.10, indicates a good agreement between these values. The accuracy of regressional models is obvious from these comparisons. Similarly Fig 5.11 and Fig 5.12 show a good agreement around mean value and are a little scattered, away from mean values. This behavior is because regression models can predict the response under a specific range and its prediction interval widens as it moves away from the mean values (Montgomery, 1991).

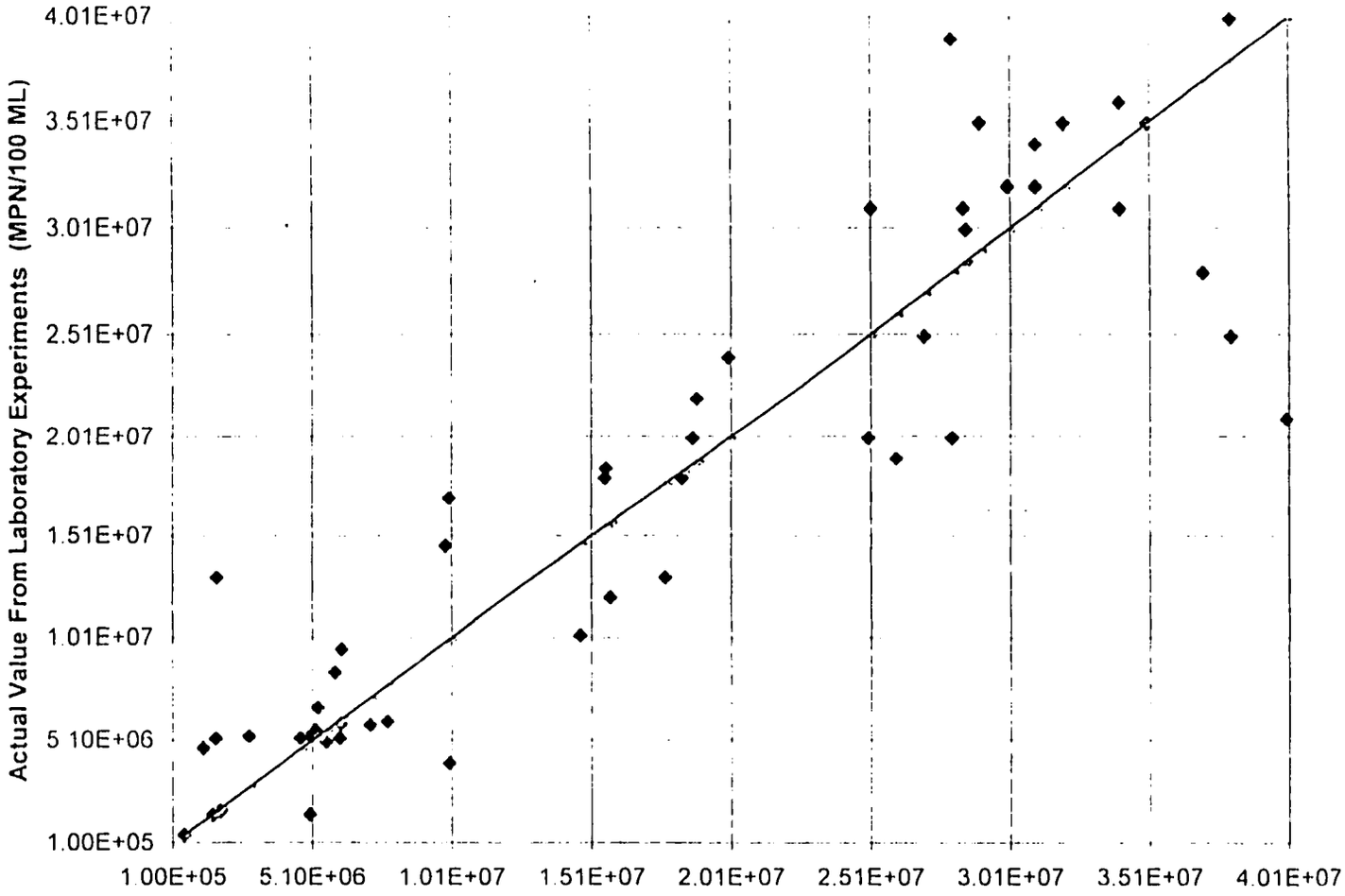


Fig. 5.10: Comparison of Predicted and Measured values of Total Coliform (Raw Sewage)

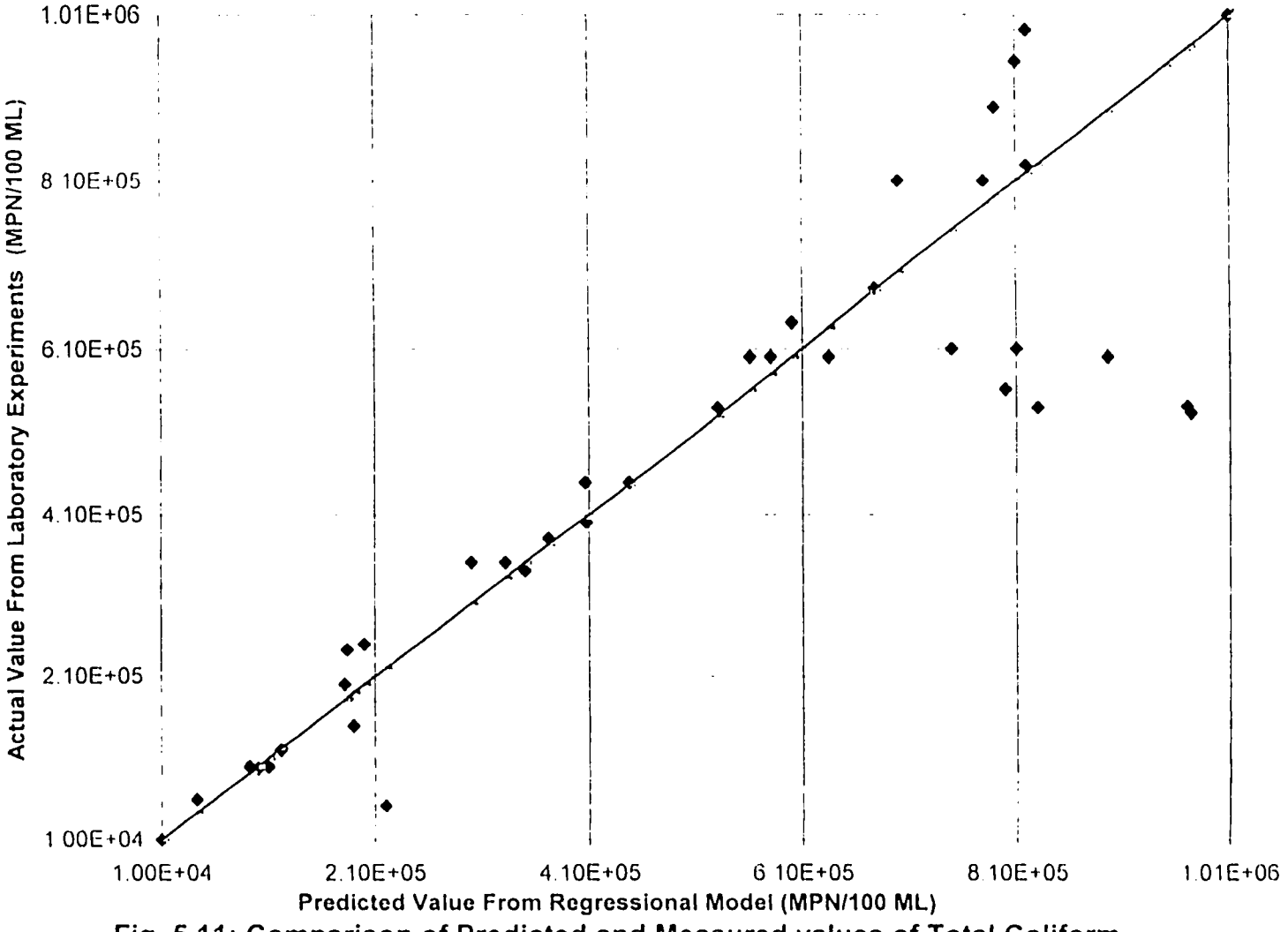


Fig. 5.11: Comparison of Predicted and Measured values of Total Coliform (Secondary Treatment)

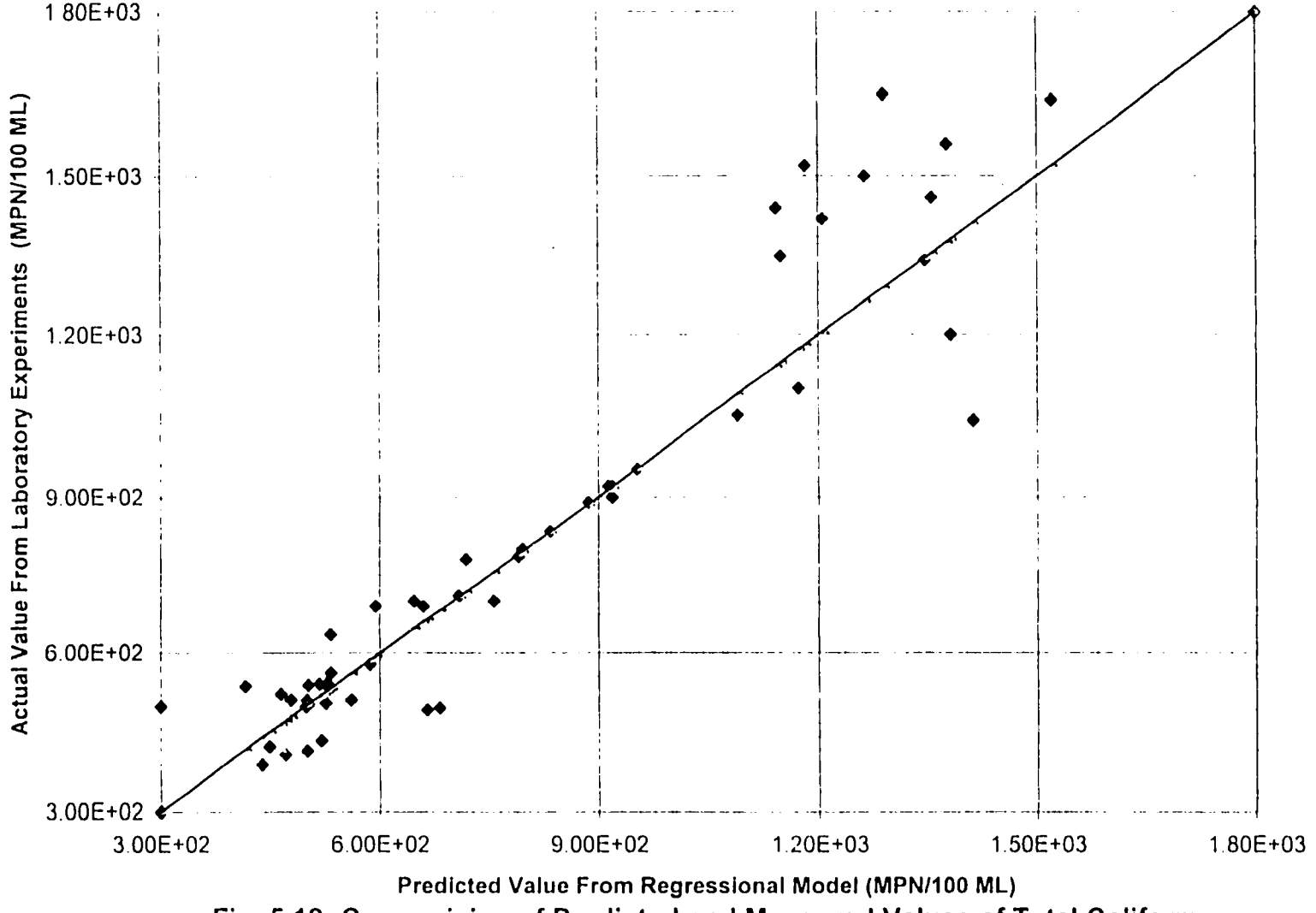


Fig. 5.12: Comparision of Predicted and Measured Values of Total Coliform (Chlorinated)

Another verification of regressional models on the basis of average percent error between the measured and predicted values (from regression models) has been made. Verification of these regressional models on the basis of average percent error is shown in Table 5.8. Values shows that the regression equations are very well compared with the actual data of microbial population density. The maximum average percent error was observed in the prediction of Standard Plate Count in the raw sewage (14.3%). The reason for this error could be due to a variety of microbial population present in the Standard Plate Count and their unknown behavior with the independent variables. Chances of errors always exist in predicting the results in running experiments or finding the best fitted distribution, (Sadiq, 1997).

The regression equations are empirical relations and there are many other factors involved in this phenomena other than flow and temperature e.g pH, sun light, spread of disease or infection in the community etc. One should keep in mind these regression equations are fitted very well between the limits of measured maximum and minimum values and these equations may give error outside these limits.

Table 5.8: Verification of the Regression Models on Average Percent Error Basis

Parameter	Raw Sewage	Sec. Effluent	Chlorinated Effluent
SPC	14.3	7.32	8.22
TC	10.90	5.60	10.9
FC	4.76	3.87	4.08
CP	5.60	6.04	3.22
C.Pr	2.90	1.77	7.53

5. 7 Combined Effect of Flow and Temperature on Microbial Population Density

It was observed that, population density of indicator microorganisms studied decreases with the increase in the wastewater flowrate. This variation may be justified by the dilution effect.

Effect of flow and temperature on microbial population density was observed during the study period. It was observed that generally an increase in the flow causes the dilution effect and an increase in temperature increases the multiplication rate of the microbial population. It is clear from Fig 5.3, to Fig 5.7, that during the high temperature and high flow conditions microbial population density almost become constant which may be due to the canceling effect of flow dilution against increasing effect of temperature (see Fig 5.3, from August to the end of summer). A similar pattern could be observed for the other indicator microorganisms. A study carried out by MetCalf and Eddy (1991), reported a lower concentration of microorganisms during high wastewater flowrate. When flowrate decreases the concentration of microorganism rise significantly. They justified the decrease in the population density of microorganism by the dilution effect of the water during high rate of water utilization season. Another study carried out by El-Sharkawi et al. (1989), reported the similar results from their study.

Similarly during high temperature and low flow condition a rapid increase in the microbial population is observed due to the supporting effect of low flow on temperature. When temperature becomes low with the relatively high flow a rapid decrease in the microbial population density is observed (see Fig 5.3, during the month of October and November). Similar effect can be observed in all the other microorganisms studied.

5. 8 Interpretation of Contour Maps

In order to see the effect of flowrate and temperature on population densities of indicator microorganisms studied, simultaneously, the contours maps were developed. Regressional models were used to developed these contour maps. Findings of this study can explained and supported with the help of these contour maps. Table 5.9 shows the data generated from Model-2 (total coliform), within the observed range of flow and temperature at the Al-Khobar sewage treatment plant. Fig 5.13 shows the corresponding contours of total coliform population density in raw sewage as a function of temperature and flowrate. The abscissa represents the variation of temperature from 25 to 34 °C and the ordinate represents the flowrate ranges from $8.0x10^4$ to 1.0510^4 m^3/day, which is within the observed range in the field.

The contour in Fig 5.13 shows that within a large bend of flowrate (from $8.0x10^4$ to $1.05x10^4$ m^3/day) the total coliform population density does not vary significantly up to a sewage temperature of 28°C. Maximum population density of

total coliform, within this range could reach up to $2.0x10^7$ MPN/100ml. When temperature of sewage remains between 31 to 32°C the total count density remains between $3.0x10^7$ to $5.0x10^7$MPN/100ml, between a wide range of flowrate, $8.5x10^4$ to $1.05x10^5$ m^3/day. It is clear from Fig 5.13 that, at any fixed flowrate temperature sensitivity of total coliform density increases with the temperature. For example, with the increase of 2 °C of wastewater temperature from 29 °C, about 50% increase in the total coliform population density observed. At the other side from 33 °C and above, only increase of one degree temperature increase the population density by the same order of 50%.

Table 5.10 and Table 5.11 were developed from Model-7 (total coliform) and Model-12 (total coliform) respectively. Fig 5.14 and Fig 5.15 show the contour maps of total coliform in secondary and chlorinated effluent, respectively. These figures clearly indicate that, at lower flowrate microbial populations increase rapidly with an increase in temperature after 28°C. Effect of flowrate, is less sensitive than temperature.

The plot of Table 5.10 is shown in Fig 5.14. It can be revealed from the figure that, at lower flow rates, sensitivity of temperature increases after 29°C. Another important behavior observed in Fig 5.14 is that the total coliform population density decreases in secondary effluent and becomes relatively less sensitive to the change in flowrate. This could be due to the large detention time

Table 5.9: Indicator Microorganism Population Data Generated From Model-2 (Total Coliform) at Different Flowrate and Temperature

Temperature	Flowrate (m^3/day)					
(℃)	80000	85000	90000	95000	100000	105000
25	5969276	2462405	358044	459195	89696	1378565
26	5150539	1503394	685142	1535071	1150792	376200
27	6483088	2695667	422957	459662	60603	1525119
28	10108961	6181265	3824381	2909073	3322912	4967364
29	16177928	12109957	9668898	8720901	9149520	10852703
30	24847842	20639597	18114364	17133676	17577075	19338989
31	36285012	31936492	29327084	28313707	28771886	30592530
32	50664582	46175787	43482205	42436139	42909098	44788472
33	68170935	63541865	60764108	59685352	60173091	62111196
34	88998102	84228758	81366827	80255381	80757900	82754735

Table 5.10: Indicator Microorganism Population Data Generated From Model-7 (Total Coliform) at Different Flowrate and Temperature

Temperature	Flowrate (m^3/day)					
(℃)	80000	85000	90000	95000	100000	105000
25	121193	55455	11992	11030	15203	1926
26	115254	46886	1684	22258	26598	12790
27	130080	59083	12143	12720	17228	2888
28	167043	93416	44737	18953	14279	29150
29	227588	151331	100914	74209	69368	84770
30	313238	234352	182196	154571	149563	165495
31	425600	344084	290189	261643	256468	272932
32	566363	482218	426584	397117	391775	408770
33	737307	650533	593161	562773	557264	574790
34	940306	850902	791791	760482	754807	772864

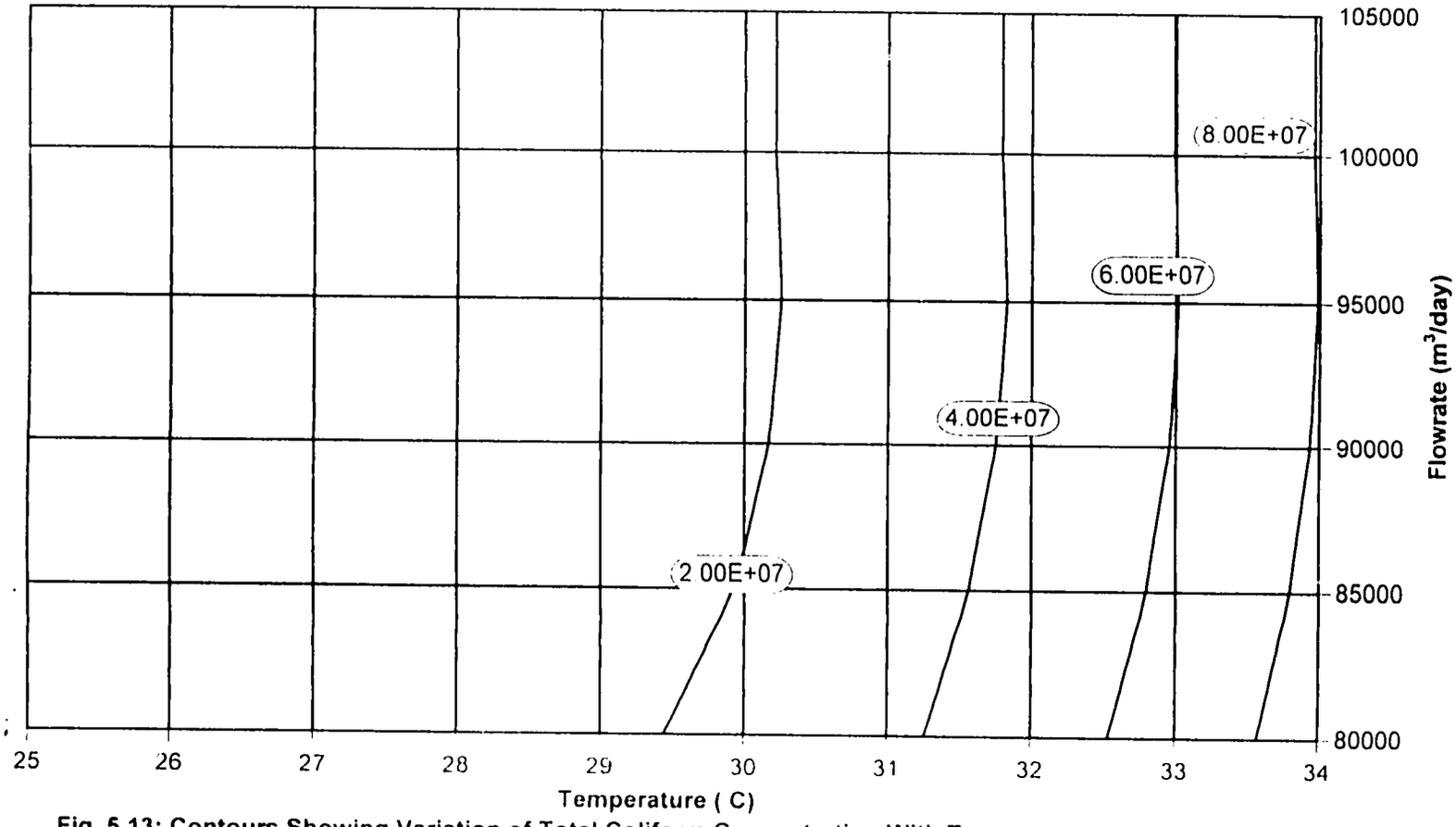


Fig. 5.13: Contours Showing Variation of Total Coliform Concentration With Temperature and Flowrate in Raw Sewage

Table 5.11: Indicator Microorganism Population Data Generated From Model-12 (Total Coliform) at Different Flowrate and Temperature

Temperature (C)	Flowrate (m^3/day)					
	80000	85000	90000	95000	100000	105000
25	546	498	473	470	488	523
26	578	528	503	500	517	554
27	621	569	543	540	558	597
28	676	622	595	592	611	651
29	744	688	659	656	676	717
30	824	767	737	734	754	797
31	919	859	829	825	847	891
32	1029	967	936	932	954	1000
33	1155	1091	1059	1055	1078	1125
34	1297	1232	1199	1195	1218	1267

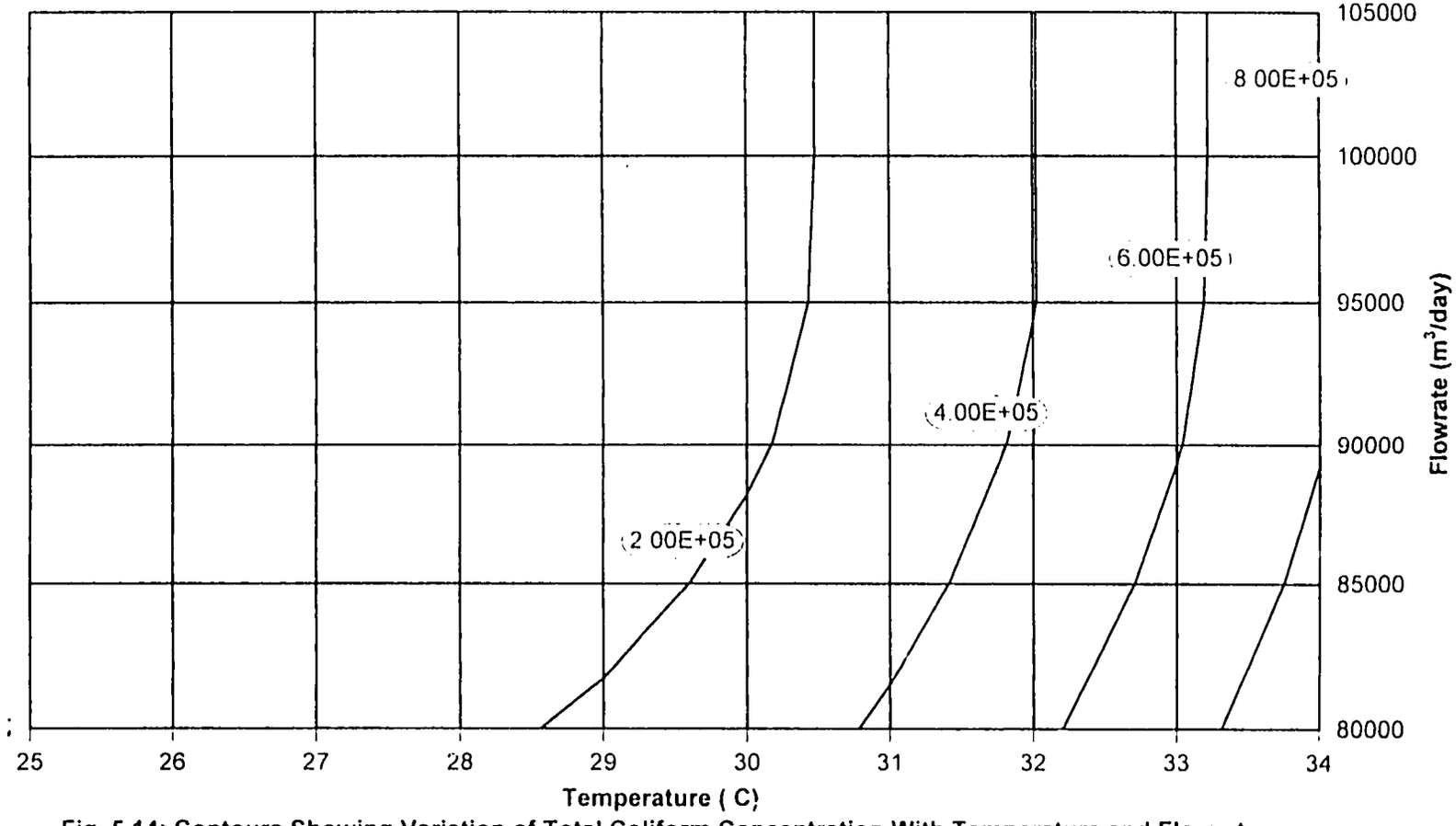


Fig. 5.14: Contours Showing Variation of Total Coliform Concentration With Temperature and Flowrate in Secondary Effluent

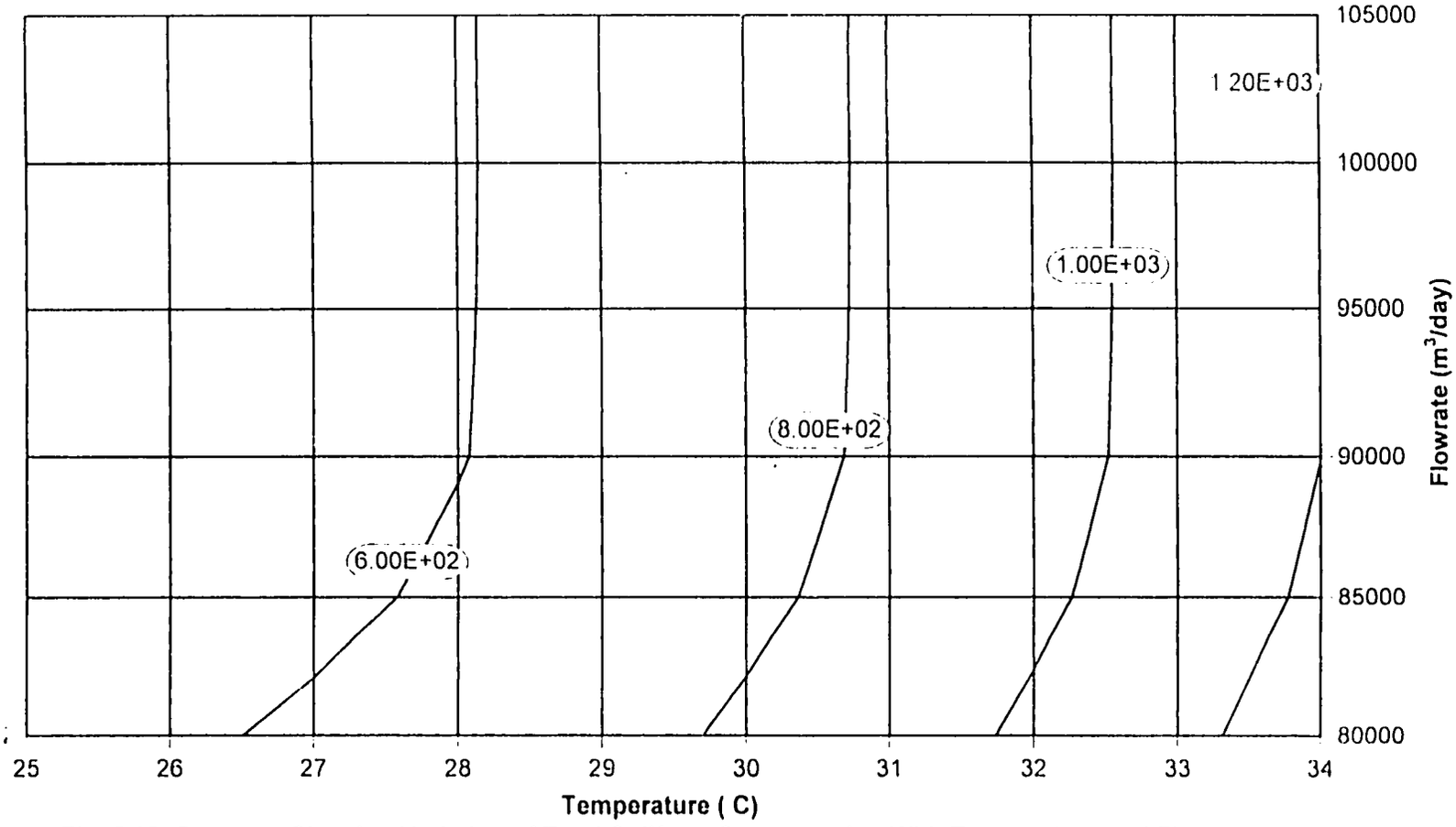


Fig. 5.15: Contours Showing Variation of Total Coliform Concentration With Temperature and Flowrate in Chlorinated Effluent

of wastewater in aeration tank and secondary clarifier. Population density of coliform, at a fixed flowrate, increases linearly with the increase of temperature (about 35 to 45% per °C increase in temperature).

The plot of Table 5.11 is shown in Fig 5.15. It is clear from the figure that, at lower flow rates, sensitivity to temperature again increases after 29°C. Similar behavior of coliform is observed in Fig 5.15, where the population density decreases in chlorinated effluent and almost becomes independent to the change in flowrate. With an increase of temperature by 1 °C total coliform population density increase linearly from 15 to 20%.

Similarly contours for Standard Plate Counts, fecal coliform, coliphage, and *Cl. perfringens* have been made and a similar trend is observed in raw sewage, secondary effluent and chlorinated effluent.

5. 9 Percent Removal of Indicator Microorganism After Different Treatments in Al-Khobar STP

Many researchers stressed the need for further study to find a more suitable microorganism as an indicator of pollution for the sake of reliability on public health and safety.

As it has been suggested by many researchers (Kwamura and Kaneko, 1986; Hirata et al., 1991), more resistant indicator microorganisms in wastewater treatment could be better indicators of pollution. In order to evaluate the

effectiveness and significance of studied indicator microorganisms, a comparison of percent removal has been made.

Percent removal of the indicator microorganisms in different effluents of the Al-Khobar STP is shown in Table 5.5. Standard Plate Count, total coliform, and fecal coliform show a higher percent removal after secondary treatment as well as after chlorination process. Percent removal of coliphage after secondary treatment is 83.62% and chlorination reduces it further by 51.96%. The high adsorption ability of coliphage with the activated sludge is responsible for its higher removal in secondary treatment (Omura et al., 1989). Similarly *Cl. perfringens* removal is 55.50% after secondary treatment and only 42.17% after chlorination (with a chlorine residual up to 0.5 mg/l).

Results presented in Table 5.5 shows that coliphage and *Cl. perfringens* could be recommended as better indicator of pollution due to their high resistance against chlorination. Similar conclusion have been made by Hirata et al. (1991). It could be inferred from Table 5.5 that, among all of indicator microorganisms studied, *Cl. perfringens* could be the better bacterial indicator of pollution through high resistance to chlorine, high conservation in environment, and least removal in secondary treatment and after chlorination.

5. 10 Variation of Microorganism Population Density in the Dry Sludge

Sludge from the sludge thickener is placed for drying over a thick bed of sand in drying beds. This dry sludge is afterward used as a fertilizer. Study of dry sludge is very important, due to the use of this sludge as a fertilizer, as it may contain a large number of pathogen, and can be a threat to the health of the community. Counts et al. (1974) suggested a number of ways for stabilization of municipal sewage sludge.

Although sludge for drying, remains over the drying beds for more than a week, analysis shows a large population densities of microorganisms. An annual average population density of total coliform and fecal coliform in the dry sludge found as $2.58x10^8$ MPN/gm dry sludge and $7.82x10^6$ MPN/gm respectively. Similarly average values of Standard Plate Counts, coliphage and *Cl. perfringens* were $3.22x10^8$ colonies/gm, $9.13x10^4$ PFU/gm, and $1.07x10^4$ colonies/gm respectively. Pepper et al. (1993) in a dry sludge study, reported similar concentration of total coliform and fecal coliform in the dry sludge as 10^8 & 10^7 per ml of sludge respectively.

Summer and winter microbial population densities obtained in this study are tabulated in Table 5.2 and Table 5.3. A comparison of population densities of indicator microorganism during summer and winter is presented in Fig 5.16. It is very clear from the tables and figure that the population density of microorganisms decreases with the increase in temperature, which is logical due

to inactivation of microorganisms by the solar radiation and heat. Van Donsel et al. (1967) found that for a 90% reduction in the fecal coliform, at the drying beds, exposed to sun, required 13.4 days during winter and only 3.3 days during the summer. Similar conclusion has been made by Pepper et al. (1993). Again a different behavior of *Cl. perfringens* is observed in the dry sludge study. Population density of *Cl. perfringens* in dry sludge is higher during summer than it is in winter ($1.17x10^3$ colonies/gm and $5.5x10^3$ colonies/gm in summer and winter respectively). This behavior is again due to the resistance of *Cl. perfringens* against high temperature which results in less inactivation during summer. Similar findings are reported in literature by many researchers (Berg et al., 1976; Pepper et al., 1993).

Student t-test analysis also performed on dry sludge data to reinforce the conclusions of the study. The results of t-test (shown in Table 5.12) for dry sludge shows that there is a significant difference in the summer and winter data for total coliform, fecal coliform and coliphage, and the values obtained during the winter are higher than the summer values which could be mainly due to the inactivation of microorganisms by the solar radiation and heat. The t-test on Standard Plate Counts (which is basically consist of large number of known and unknown microorganisms and may contain some more temperature resistant or spore forming microorganisms) and *Cl. Perfringens* (which is highly temperature resistant spore-forming anaerobic bacteria) shows that there is no on Standard Plate Counts (which is basically consist of large number of known and unknown

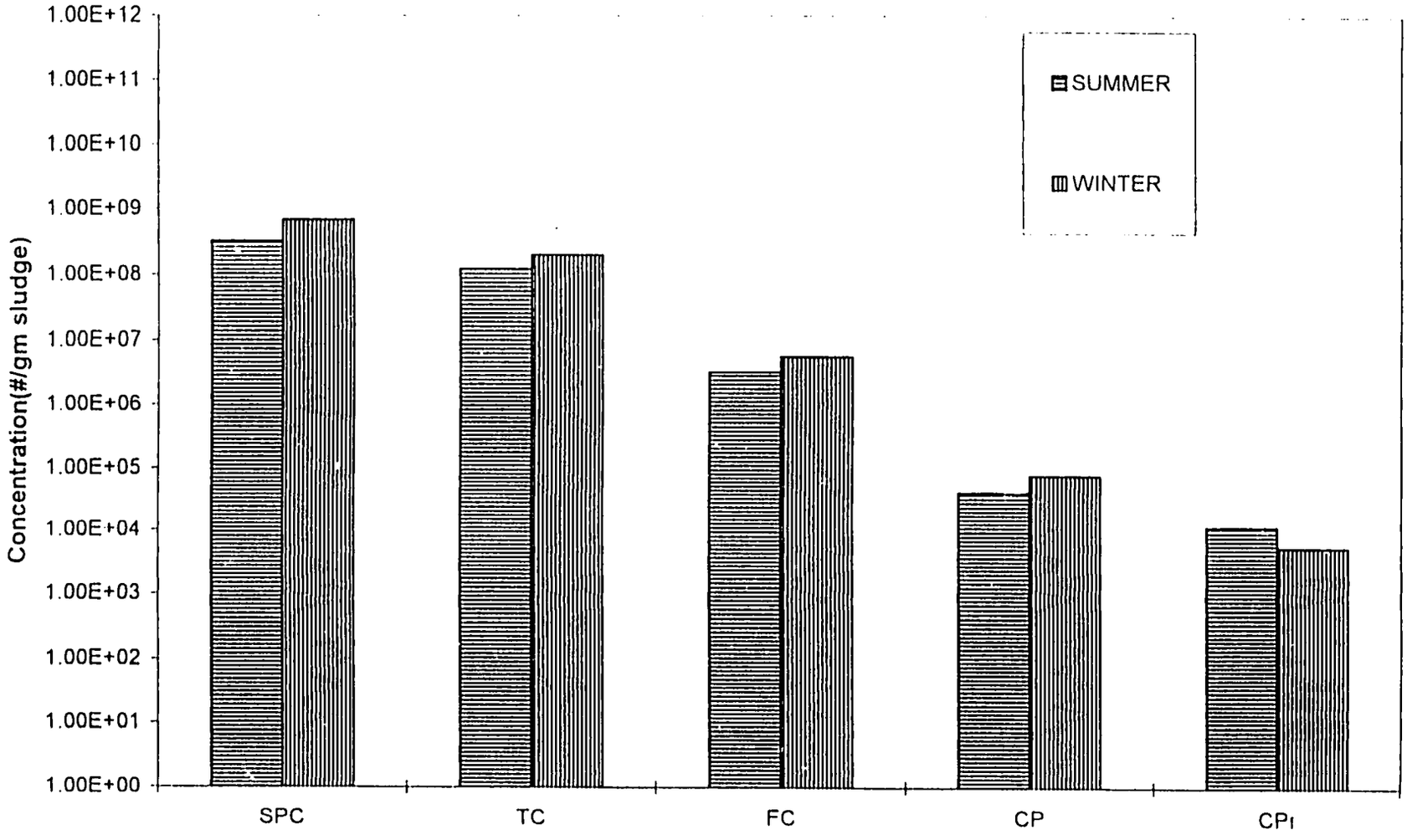


Fig. 5.16: Comparision of Different Indicator Microorganism Population in Dry Sludge of Al-Khobar STP (Summer and Winter)

Table 5.12: Statistical Comparison of Summer and Winter Microbial Population Density using t-test @ 95% Confidence Interval

		SUMMER DATA			WINTER DATA							
Sample	Microb	Sample Size (n_1)	Mean (Y_1)	Variance S_1^2	Sample Size (n_2)	Mean (Y_2)	Variance S_2^2	t_{cal}	t_{tab}	D.F	Null Hypoth	Inference
Dry	SPC	30	1.21×10^{8}	4.98×10^{16}	22	1.60×10^{8}	5.94×10^{16}	-0.5883	2.0178	43	Cannot Reject Ho	summer = winter
Sludge	TC	30	1.23×10^{8}	1.94×10^{16}	22	2.93×10^{8}	4.85×10^{16}	-3.1929	2.0357	33	Reject Ho	summer < winter
	FC	30	5.27×10^{6}	1.31×10^{13}	22	9.52×10^{7}	3.04×10^{13}	-3.1516	2.0336	34	Reject Ho	summer < winter
	CP	30	4.13×10^{4}	9.89×10^{8}	22	1.16×10^{5}	6.12×10^{9}	-4.2418	2.0560	26	Reject Ho	summer < winter
	C.Pr	30	1.37×10^{4}	1.74×10^{8}	22	9.49×10^{3}	5.23×10^{7}	1.4597	2.0136	47	Cannot Reject Ho	summer = winter

microorganisms and may contain some more temperature resistant or spore forming microorganisms) and *Cl. Perfringens* (which is highly temperature resistant spore-forming anaerobic bacteria) shows that there is no significant difference in summer and winter data and their population density in dry sludge is independent of seasonal changes. Mitchell (1974), explained this behavior and stated that, spore forming property increase the survival rate of microorganisms because by this protective spore they can resist more adverse environmental conditions. *Cl. perfringens* does not multiply and is incapable of proliferating even in nutrient-rich sewage, because of its extremely high nutrient requirements (Boyd et al., 1948; Fuchs and Bond, 1957). Therefore there is no significant difference in population density observed during summer and winter seasons. Sorber et al. (1984) stressed on more research in this area due to the paucity of available literature.

CHAPTER 6

6. SUMMARY AND CONCLUSIONS

This study was aimed to evaluate the Al-Khobar wastewater treatment plant from microbial point of view and see the seasonal variations in the microbial population densities in different effluents of the plant and dry sludge.

During this study variation in the temperature of wastewater arriving at the treatment plant were observed and found to range between 34.6°C and 23.5°C, during summer and winter respectively. Flowrate was observed to range from 124,880 m^3/day to 82,050 m^3/day, during summer and winter respectively.

A prominent variation in the microbial population density was observed with the change of season. Increase in the temperature during summer shows an increase in the population densities of microorganisms in different effluents of the wastewater treatment plant. The mean population densities of Standard Plate Count, total coliform, fecal coliform, coliphage, and *Cl. perfringens* in the raw sewage during summer were observed as 3.01×10^{11} colonies/100 ml, 3.11×10^{7} MPN/ 100 ml, 9.81×10^{7} MPN/ 100 ml, 3.73×10^{2} PFU/100 ml and

$2.58x10^2$ colonies/100 ml respectively. Similarly these population densities decreased during winter and were observed as $7.02x10^8$ colonies/100 ml, $1.76x10^6$ MPN/ 100 ml, $2.73x10^6$ MPN/ 100 ml, $1.52x10^2$ PFU/100 ml and $1.92x10^2$ colonies/100 ml respectively. The mean population densities of these microorganisms were reduced by more than two log order after secondary treatment. Observed densities for different indicator microorganisms were $3.36x10^8$ colonies/100 ml, $3.67x10^7$ MPN/ 100 ml, $1.27x10^8$ MPN/ 100 ml, $4.62x10^1$ PFU/100 ml, and $2.07x10^2$.colonies/100 ml respectively during summer. During winter after secondary treatment mean densities were observed to be $8.47x10^6$ colonies/100 ml, $1.66x10^4$ MPN/ 100 ml, $1.63x10^4$ MPN/ 100 ml, $7.95x10^1$ PFU/100 ml, and $1.53x10^2$ colonies/100 ml respectively.

Chlorine gas is being used at the Al-Khobar wastewater treatment plant as a disinfectant. During this study residual chlorine was observed in the range of 0 to 0.5mg/l. In the chlorinated effluent the microbial population densities were reduce by more than three log order. Population densities of these indicator microorganisms during summer were observed as $2.08x10^3$ colonies/100 ml, $1.81x10^3$ MPN/ 100 ml, $9.85x10^2$ MPN/ 100 ml, $9.46x10^1$ PFU/100 ml and $1.38x10^2$ colonies/100 ml respectively. During winter these densities reduced to $2.20x10^6$ colonies/100 ml, $3.97x10^2$ MPN/ 100 ml, $3.78x10^2$ MPN/ 100 ml, $3.62x10^1$ PFU/100 ml and $1.23x10^2$ colonies/100 ml respectively.

The annual average microbial population densities were observed in the Al-Khobar wastewater treatment plant. Standard Plate Counts initially

measured in the raw sewage were $4.71x10^{10}$ colonies/ 100 ml and reduced to $7.16x10^{7}$ colonies/100 ml (99.85%) in the secondary effluent. Finally after the chlorination, density reached up to $9.23x10^{6}$ colonies/ 100 ml (86.27%). Decrement of 99.22% in the total coliform was observed in the secondary effluent and then 99.72% reduction takes place in the chlorinated effluent. Fecal coliform reduce to 98.78% in the secondary effluent and then 99.62% reduction takes place in the chlorinated effluent. Coliphage reduce to 83.62% in the secondary effluent and then 51.96% reduction takes place in the chlorinated effluent. *Cl. perfringens* counts decrease to 55.50% in the secondary. *Cl. perfringens* show more resistance against chlorination and reduce only by 42.07% in the chlorinated effluent.

Contour maps were found to be very useful in observing the effect of flowrate and temperature simultaneously on the microbial population densities. Contour map showed that the population densities of studied microorganisms within a large bound of flowrate does not vary significantly at lower temperature (about 26 to 28°C). At lower flowrates microbial population increases rapidly with the increase in temperature after 28°C. Chlorinated effluents are less sensitive to the effect of flowrate. In the chlorinated effluents *Cl. perfringens* becomes almost completely independent of flow and more sensitive to change in temperature. Increase of about 4°C, bring 15 to 20% increase in the *Cl. perfringens* population density.

Multiple non-linear stepwise regression was used to correlate microbial population density data of all studied indicator microorganisms with the variations in flow and temperature. Verification of regressional models based on a plot of actual versus predicted data and on average percent error between measured and predicted values are compared very well. Only Standard Plate Counts in raw sewage have higher percent error (14.3%).

Change in the microbial population densities in dry sludge were observed and it was found that *Cl. perfringens*, which is a spore forming anaerobic bacteria, can survive high temperatures and has higher population density during summer than in winter. All other studied microorganisms have lower population densities during summer compared to winter.

The t-test analysis for Standard Plate Count and *Cl. perfringens* in dry sludge shows that, there is no significant difference in summer and winter mean values, and their population density is independent of seasonal changes.

Removal efficiencies of all studied indicator microorganisms were determined to see the behavior of these microorganisms against different treatments. Standard plate counts, total coliform, and fecal coliform were removed in high percentage (99% to 99.9%) after secondary treatment. Coliphage was removed up to 83.6%, while *Cl. perfringens* removed only up to 55.5%. After chlorination Standard Plate Counts, total coliform, and fecal coliform were removed up to 99.7% while coliphage reduced up to 52%. Similarly *Cl. perfringens* was removed only by 42%.

Conclusions

Following are the specific conclusions made in this study

1. Population density of the indicator microorganisms studied increases with an increase in wastewater temperature and decreases with an increases in the wastewater flowrate.
2. After secondary treatment Standard Plate Count, total coliform, and fecal coliform were removed in high percentage (99% to 99.9%) and coliphage removed by 83.6%, while *Cl. perfringens* was removed by only 55.5%.
3. After chlorination Standard Plate Count, total coliform, and fecal coliform were removed up to 99.7%, while coliphage reduced by 52% and *Cl. perfringens* was removed by only 42% and showing a high resistance against chlorination.
4. Effect of chlorination on microbial population is more during summer. Standard Plate Count shows high removal after chlorination during summer (99.99% in summer and 74.1% in winter). But *Cl. Perfringens* shows least removal after chlorination during summer (14.91% in summer and 19.87% in winter).
5. The t-test analysis at confidence level of 95% shows, the mean population densities of all the indicator microorganisms studied is high in summer as compared to it is in winter.
6. For the dry sludge t-test analysis at 95% confidence level shows that, mean population density of total coliform, fecal coliform, and coliphage is less

during winter as compared to summer, but Standard Plate Count and *Cl. perfringens* population densities had no significant difference.

7. Verification of Regressional Models for all studied indicator microorganisms showed a good agreement with actual data. A maximum 14.3% error was observed in the model of Standard Plate Count in raw sewage.

CHAPTER 7

7. RECOMMENDATIONS

On the basis of this study and conclusions derived from the results following recommendations can be made.

1. During this study, the experiments were conducted without any plan of design of experiments and many important set of readings are missing. To enhance the accuracy of the analysis, data during the rainfall and wastewater temperature in each treatment unit must be obtained. To develop more precise models these factors must be included in the regression equations.
2. Presence of small quantity of heavy metals and toxic materials can alter the treatment efficiency of a wastewater treatment plant due to the adverse effect of these materials on microbial populations. Study in the future may include these parameters to get a clearer picture of the treatment performance of the plant.

3. As the dry sludge produced in the Al-Khobar wastewater treatment plant is being used as a fertilizer and which contains a large number of bacteria and viruses, a more extensive study on dry sludge is required to ensure the protection of community from spread of any disease due to the pathogens present in the dry sludge.

. Batch dumping of industrial waste in the Al-Khobar wastewater treatment plant is in practice, the effect of these shock loads on the plant performance should be studied. This study could be accomplished by pre-plan of design of experiments to ensure the collection of all important relevant data.

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