# MICROBIOLOGY OF WATER FROM THE TUCSON SEWAGE TREATMENT PLANT FACILITIES INCLUDING A PILOT FILTER

by

Jaime Julio Mendiola

---

A Thesis Submitted to the Faculty of the

DEPARTMENT OF MICROBIOLOGY AND MEDICAL TECHNOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

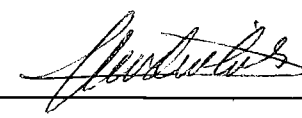
THE UNIVERSITY OF ARIZONA

1967

## STATEMENT BY AUTHOR

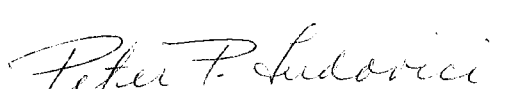
This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: 

## APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:



PETER P. LUDOVICI

5/17/67

Date

## ACKNOWLEDGMENTS

The author wishes to express his thanks and appreciation to Dr. Peter P. Ludovici for his advice, suggestions and patience in the direction of this thesis, review of manuscript, and help in overcoming language problems. Special thanks are extended to Dr. Wayburn S. Jeter and Dr. Robert J. Janssen for their review and constructive criticism.

The writer is greatly indebted to the late Dr. Kenneth F. Wertman for his guidance and generous help, and to the Committee On International Exchange of Persons and Universidad Autonoma de Guadalajara for granting him a fellowship at the University of Arizona. Special note of appreciation to Mr. Fernando Lopez for drawing of graphs, Mrs. Alex Hodge and Mr. Cliff Butler for their technical guidance and to Mr. and Mrs. Henry Borquez for their uncommon altruism.

Words are not enough to express the acknowledgment the author wishes to extend to his wife, Maria, for her patience and sacrifice.

To her and our children, I dedicate this work.

TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF ILLUSTRATIONS

## ABSTRACT

A research project is being conducted to demonstrate the safety of renovated wastewater for the future recreational water supply to Tucson. As a part of the project, microbiological studies have been done to determine which type of sewage treatment would be the best to charge a pilot filter, based on the total numbers of bacteria, coliform density, presence of Salmonella, Shigella, and enteroviruses in water samples taken at different effluents at the Tucson Sewage Treatment Plant. Samples were tested according to standard methods and the results obtained are the substance of this thesis.

Activated sludge treatment reduced 96.1% of the total bacteria and 88.6% of the coliforms as compared with trickling filter treatment which reduced only 65% of the total bacteria and 39.8% of the coliforms. Holding pond reduced 77% of the total bacteria and 85% of the coliforms. It is concluded that activated sludge effluent should be used to charge the pilot filter. This pilot filter was effective in removing 99.6% of the total bacteria and 99.9% of the coliforms.

No Salmonella or Shigella species were found under the conditions of this experiment. Enteroviruses were detected in two out of three samples.

# INTRODUCTION

## Microbiology of Water and Sewage

Pollution of natural water transforms it into sewage. Pollution occurs by: 1) water-borne wastes from domestic sources, including human excrements, and 2) water-borne wastes from industrial sources--everything discharged by factories. Atmospheric, ground and surface waters are also part of sewage (Pelczar and Reid, 1965).

Natural water itself contains a great number of different kinds of bacteria. Briefly, they are: 1) higher bacteria (Chlamydobacteriales, sulfur, iron and other forms), 2) Caulobacteria, 3) spiral forms, 4) bacilli (including pigmented forms such as Serratia marcescens and various non-pigmented forms such as flourescent bacteria like Pseudomonas flourescens, sulfur bacteria, thermophiles, and aerobic spore-forming bacilli), 5) cocci, such as Sarcina lutea (pigmented), and Micrococcus aquatiles y M. candicans (non-pigmented), 6) nitrogen-fixing bacteria (Azotobacter aquatile in particular), and 7) nitrifying bacteria--both Nitrosomonas and Nitrobacter.

As well as bacteria, natural water contains such microorganisms as algae, fungi, and protozoa. Spirogyra, Volvox, Cosmarium, are instances of the former, Gallionella and Sphaerotulus among the fungi, and Amoeba, Vorticella, Paramaecium among the latter.

Besides protozoa, other small animals can be found, such as Rotifera and Crustacea.

By contact with air, soil and human or animal excreta, the water is contaminated with many other microorganisms, such as pathogenic bacteria, molds, yeasts, protozoa, and helminths as well as viruses (Burrows, 1963).

Sewage itself is an excellent microbiological medium with all the inorganic and organic materials necessary for good microbiological growth. In general, we could say that such water contains almost every type of microorganism. The number and types of microorganisms depend on the environment and the available oxygen. Strict aerobic bacteria have difficulty surviving in stale sewage because of the lack of oxygen but can survive in a spore stage; similarly, some fungi and protozoa exist in a spore or cyst stage, but where the conditions are favorable, for instance at the sewage-air interface, some of the lower forms can grow, by attaching themselves to sewers (McKinney, 1962).

Contamination with both human and animal excreta results in the presence of protozoa such as Entamoeba histolytica but more important is the presence of pathogenic bacteria and viruses of the same origin. Members of the family Enterobacteriaceae are the most important and the isolation of Salmonella and Shigella as well as others of less importance has been reported. Salmonella typhosa has been isolated from sewage-contaminated irrigation water (Dunlop, Twedt, and

Wang, 1951). Several types of Salmonella were found in irrigation water (Dunlop et al, 1952). Collet et al., (1953) isolated the same bacteria from well water and Bloom, Mack, and Mallmana (1958) from sewage.

Enterococci and colibacilli, from the standpoint of public health hazards have been considered as indicators of fecal contamination from sewage when found in soils and vegetables (Heukelekian, 1953). This is similar to standards set by the U. S. Public Health Department for drinking water. Other members of the enteric family pathogenic for man have been found in polluted waters (Proteus, Citrobacter, Klebsiella-Aerobacter). In addition to enteric organisms several other pathogenic bacteria have been recovered from sewage, including Pseudomonas aeruginosa (Ringen and Drake, 1952) and tubercle bacilli (Heukelekian and Albanese, 1955; Jensen, 1954; Kelly, Clarck, and Coleman, 1955).

Raw sewage is also an excellent medium for the maintenance of viruses. Poliomyelitis, cosackie, echo, influenza, adeno, and hepatitis viruses are among those which have been found (Mack et al., 1958; Chang et al., 1958; Kabler, 1959).

As the result of microbial activity in sewage, substances are produced which cause sewage odors. Facultative and strictly anaerobic bacteria degradate proteins and reduce sulfates, giving as end products skatols, mercaptans, butyric acid, aldehydes, and volatile compounds.

Sulfate reducing bacteria degrade fats and produce hydrogen sulfide as an end product.

## Sewage Treatment Effluents

There are two basic methods for the treatment of sewage: 1) aerobic, and 2) anaerobic. The aerobic system includes activated sludge, trickling filter, and oxidation pond treatments. Since these three treatments are pertinent to the present work, they will be discussed here.

The functional principle is the same for all of them: the decomposition of organic compounds by living organisms which use the energy liberated during the process for their own metabolism. These three methods are considered best for reducing the organic content of wastewater.

Activated sludge is formed by a mass of active microorganisms that develop by aerating degradable waste. Wastewater enters the aeration tank, mixes with a portion of return sludge and is aerated as long as it flows. The organic matter is stabilized aerobically by microorganisms and flows into the sedimentation tank. Activated sludge returns to the aeration tank to repeat the process. Activated sludge is made up of bacteria, fungi and protozoa, rotifera and nematodes. The most important of these are bacteria, which contribute directly to the stabilization of organic matter in the wastes being treated. Many

kinds of bacteria are contained in activated sludge and the genera which predominate depend on the nature of compounds in the wastes. Proteolytic bacteria like Flavobacterium and Bacillus predominate when wastewater is rich in proteinaceous material, while Pseudomonas species predominate when hydrocarbons or carbohydrates are present.

The trickling filter is also an aerobic biological waste treatment system and perhaps the most widely used. It may be defined as a "fixed bed system over which sewage or waste is intermittently or continously discharged and contacted with biological films on the filter media. . ." (Eckenfelder and O'Connor, 1964). It consists of a "pile of rocks" over which sewage slowly trickles. The liquid is distributed uniformly over the surface of the stones from a rotatory distributor. The liquid forms a thin layer over the surface of the stones and passes to lower layers of stones. The liquid absorbes oxygen and gives the microorganisms an opportunity to carry out aerobic metabolism. The removal of organic matter by this trickling filter is a function of the microorganisms present, the concentration of organic material, the rock size, the time of retention of the liquid in the filter, and the temperature. In the trickling filter there are bacteria, fungi, algae, protozoa and small metazoa. Bacteria predominate including Bacillus, Desulfovibrio, Pseudomonas, Alcaligenes, Flavobacterium, and Micrococcus, as well as members of the family Enterobacteriaceae (McKinney, 1962).

An oxidation pond is a large shallow pond in which wastewater is held for a period of time to permit decomposition of organic matter. The decomposition processes are both aerobic and anaerobic. They are brought about by bacteria, algae, and protozoa. Briefly, the bacteria metabolize the organic matter aerobically, yielding bacterial protoplasm, carbon dioxide, and water. Algae take up the carbon dioxide produced by bacteria, water and inorganic minerals and convert them into algal protoplasm, by using energy from the sunlight. When carbon dioxide is reduced, oxygen is released. This released oxygen in addition to oxygen from surface aeration is used by the bacteria to stabilize the organic matter. The kinds of microorganisms that must be present to carry out this function are variable and depends upon the concentration of organic wastes and the physical design of the oxidation pond. Predominant bacteria are Pseudomonas, Flavobacterium, and Alcaligenes.

The presence of algae depends upon the type and concentration of nutrients. Euglena and Chlorella predominate where the nutrient level is high. Filamentous green algae exist when the nutrient level drops off. Some of these are Spirogyra, Vaucheria, and Ulothrix. The presence of protozoa also depends on the nutrients present. High organic wastes stimulate growth of Chilomonas, Colpidium, Paramaecium, Glaucoma, and Euplates. As the bacterial population

decreases, Vorticella and Epistylius grow. Daphnia and Rotaria usually exist when organic matter is low.

## Sewage Reclamation

According to McGahuey (1957) sewage reclamation is the "purposeful upgrading of the quality of sewage with the intent of making it re-usable by agriculture, industry or the public." It was not until the third decade of this century that the use of reclaimed water was initiated. In 1930, Goudey demonstrated that sewage could be applied safely to the ground water by spraying it on the surface.

In 1949, Arnold, Hedger and Rawn found it technically possible to recharge ground water with treated sludge. However, the belief that the suspended matter in sewage could cause clogging, the fear that pathogenic bacteria might travel long distances in ground water, and the recent knowledge about the survival of viruses in sewage, as well as psychological and economical problems, delayed progress in the use of reclaimed water.

In 1957, (McGahuey, 1957) it was shown in California that sewage could be applied to soil and that intestinal bacteria were removed from the water within the first four feet of travel through the soil. In 1954, (McGauhey, 1957) irrigation of crops and pastures of 74 communities and 32 private or public institutions in the United States were carried out with sewage effluents. In view of these experimental

studies, it was decided at the conference on Waste Water Reclamation held in Berkeley in 1956, (McGauhey, 1957) that reclamation of water is technically and economically feasible and that there exist no undue legal obstacles to the re-use of water from sewage. In 1957, McGauhey stated: ". . .in general, it might be said that we have been slow to re-use waste water because we have just not been sufficiently interested in doing so, or in solving problems necessary to make it possible, as long as we can get along without it. . ."

In recent times we have many examples of the use of water reclamation for recreational purpose. Canyon Ferry Reservoir in Montana, provides sight-seeing, fishing, camping, picnicking, swimming, boating, and waterskiing for vacationists. Jackson Lake, in Wyoming, is enjoyed by nearly a million vacationers each year. Through the years, many reclamation lakes have been built in the mountain areas near Salt Lake City. These lakes not only play an important part in the transformation of desert land into farms, industries, and cities, but they also provide attractive recreational opportunities. In addition, Glen Canyon Dam (Arizona-Nevada), Grand Coulee Dam (Washington) and Folsom Dam (California) are other examples of man's ability to harness reclaimed water for recreational and industrial purposes.

Statement of the Problem

Two years ago, on June 21, 1965, a research project was authorized by U. S. Public Health Service for the city of Tucson, Arizona, entitled "Tucson Wastewater Reclamation Study" to ". . . demonstrate the chemical, microbiological, and virological safety and aesthetic acceptability of including renovated wastewater as a dependable and substantial portion of the future water supply for Metropolitan Tucson. . ." (First Annual Report. Tucson Wastewater Reclamation Project to Federal Pollution Control Administration, 1966). The objectives of this project are: 1) to demonstrate the feasibility of using sewage effluent for recreational purposes such as fishing, boating, and swimming; 2) to construct and operate a pilot plant scale sand filtration plant for the purpose of establishing full scale design characteristics; and 3) to demonstrate the safety of renovated wastewater by conducting a series of microbiological, virological, and other tests in the pilot plant and by systematic sampling of ground water in areas now recharged with wastewater (First Annual Report, Tucson Wastewater Reclamation Project, 1966).

The pilot filter consists of a series of four filter basins shaped like inverted pyramids measuring a combined total of 250 ft in length, 125 ft in width and 18 ft in depth. The filtration medium consists of sand, gravel and native soils. Treated sewage effluent from the plant is

fed into these units, allowed to filter by gravitational flow and the resultant clear, reclaimed water is pumped to a series of four fishponds.

The microbiological studies are designed to determine 1) the standard count of bacteria, 2) the number of coliforms, 3) the presence of Salmonella or Shigella, and 4) the presence of enteroviruses in various treated sewage and pilot filter effluents at the Tucson Sewage Treatment Plant.

The purpose of my particular phase of the study was to investigate which of the treated sewage effluents might best be used, in the future, for charging the pilot filter. The basis for selection would depend on which treated sewage effluent contained the lowest number of total bacteria, coliforms, Salmonella or Shigella, and enteroviruses. Based on the results cited in the literature, activated sludge effluent was used initially to charge the filter. It was also of interest to study the bacterial content of the pilot filter influent and effluent water. The results of this phase of the project are the substance of this thesis.

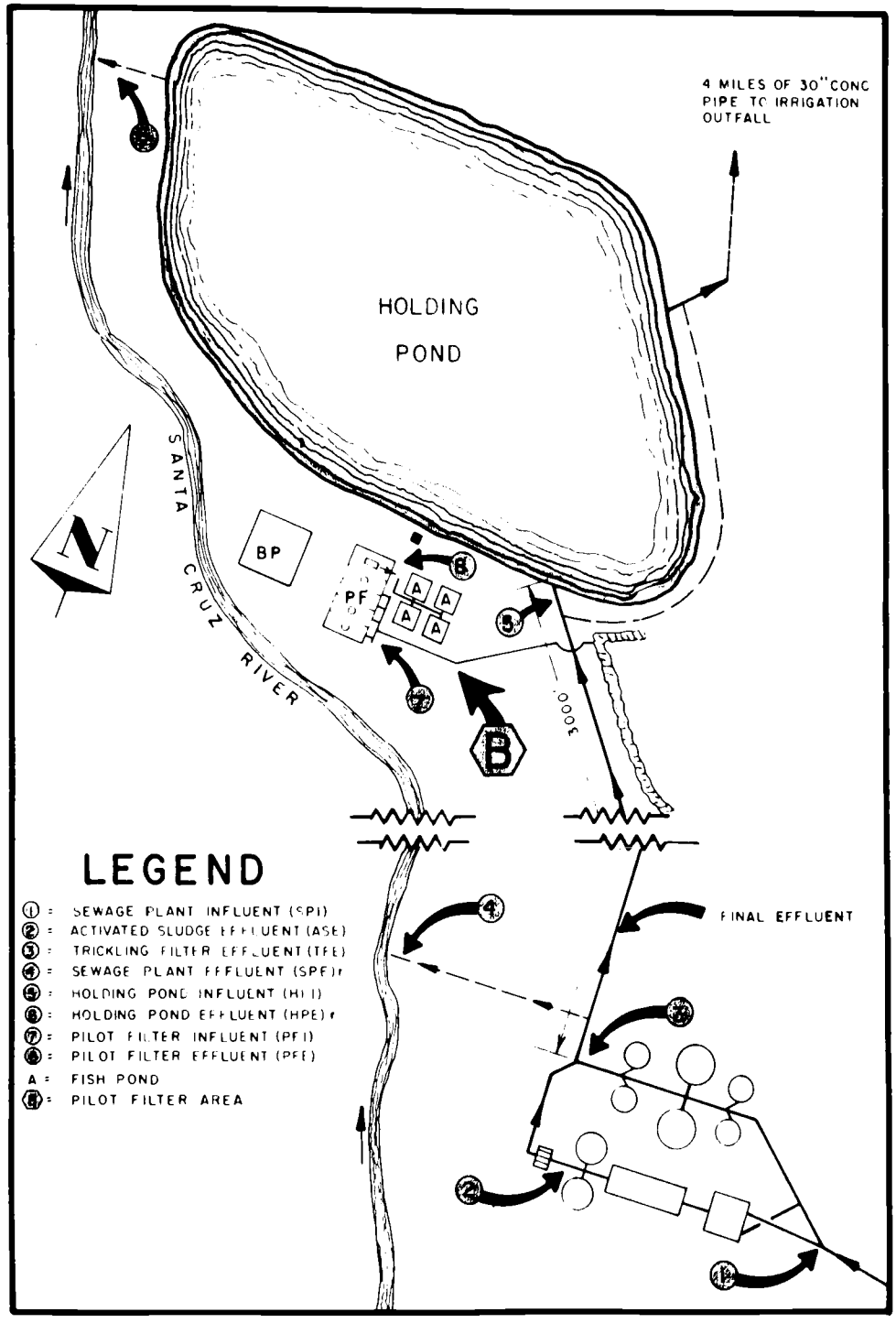
# MATERIAL AND METHODS

## Sample Sites and Methods of Collection

Water samples were obtained from seven different sources at the Tucson Sewage Treatment Plant. These included: Sewage Plant Influent (SPI), Activated Sludge Effluent (ASE), Trickling Filter Effluent (TFE), Holding Pond Influent (HPI), Holding Pond Effluent (HPE), Pilot Filter Influent (PFI), and Pilot Filter Effluent (PFE). The location of these sampling points in respect to the overall treatment plant is presented in Fig. 1. During the course of this study, chlorination of treated sewage effluents was discontinued because of a plant expansion program. Also, activated sludge effluent was loaded only into basin one of the pilot filter. Thus, this water was filtered through approximately 190 ft of horizontal and 15 ft of vertical sand, gravel, and native soil before it became pilot filter effluent.

Samples for standard bacterial counts, coliform density, and Salmonella-Shigella determinations were obtained in sterile wide-mouth amber jars with plastic screw-caps. They were immediately taken to the laboratory and refrigerated until they were processed which was within three hours from the time of collection. Virus samples were collected in sterile 1 gallon stainless steel pressure tanks, and similarly handled.

Fig. 1.--Schematic Drawing of Tucson Sewage Treatment Plant.


4 MILES OF 30"CONC PIPE TO IRRIGATION OUTFALL
HOLDING POND
SANTA CRUZ RIVER
BP
PF
A
A
A
A
3000'
B
FINAL EFFLUENT
LEGEND
① = SEWAGE PLANT INFLUENT (SPI)
② = ACTIVATED SLUDGE EFFLUENT (ASE)
③ = TRICKLING FILTER EFFLUENT (TFE)
④ = SEWAGE PLANT EFFLUENT (SPE)
⑤ = HOLDING POND INFLUENT (HPI)
⑥ = HOLDING POND EFFLUENT (HPE)
⑦ = PILOT FILTER INFLUENT (PFI)
⑧ = PILOT FILTER EFFLUENT (PFE)
A = FISH POND
B = PILOT FILTER AREA

Standard Plate Counts

Each water sample was diluted and tested by adding 1 ml to duplicate poured Difco-plate count agar dishes and incubated at 35 C for 48 hr, according to Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WPCF, 1960). Average colony counts from two plates containing 30 to 300 colonies were used to determine the total standard bacterial content of each water sample. Colony counts were done on a New Brunswick colony counter.

Coliform Density

The multiple tube method was used according to standard procedures (APHA, AWWA, WPCF, 1960) to determine coliform density. Three dilutions of 5 tubes each in the usual geometric series were employed. Lactose medium was used, and prepared according to standard procedures (APHA, AWWA, WPCF, 1960). Any amount of gas present within 48 hr after inoculation was considered a positive reaction (lactose fermentation). At least one tube from the highest positive dilution was used to confirm the presence of coliforms. This was done by transferring a loop to brilliant green lactose bile broth. The presence of acid and gas after 48 hr incubation at 37 C constituted a positive reaction. The most probable number (MPN) of coliforms was determined according to standard tables of MPN (APHA, AWWA, WPCF, 1960).

Data obtained were corrected for bias by the correction factor for five fermentation tubes, which is 0.81 (Thomas, Woodward and Kabler, 1956).

Salmonella-Shigella Determination

Attempts to detect the presence of these bacteria were made by the following methods:

1) The water sample was centrifuged at 6,000 rpm for 30 minutes and the sediment inoculated into enrichment medium (selenite or tetrathionate broth).

2) One hundred ml of water were filtered through a 0.45 $\mu$ Millipore membrane and the 47 mm membrane was placed on an absorbent pad impregnated with bismuth sulfite broth in 60 mm plastic petri dish (Kabler and Clark, 1952).

3) One-half to one gallon of water was filtered through a 0.22 $\mu$ Millipore membrane and the 142 mm membrane was cut into 55 mm circular sections which were placed on absorbent pads impregnated with bismuth sulfite broth in a 60 mm plastic petri dish.

4) One hundred ml of water were filtered through 0.45 $\mu$ Millipore membrane and the 47 mm membrane was placed in a thioglycollate broth tube for 20 minutes at 37C, removed, and placed in selenite broth tube overnight.

5) One hundred ml of water were filtered through a 0.45 $\mu$ Millipore membrane and the 47 mm membrane was placed in a selenite broth tube and incubated at 37 C for 18 hr.

6) Cotton cheesecloth pads made from eight strips of four inches in width by 36 inches in length, cut and folded to give pads four by ten inches in shape and one inch thick, were immersed for 48 hr to one week at various sites at the sewage plant. These pads were collected in plastic bags and returned to the laboratory where the water was squeezed out of the pad and subsequently centrifuged at 6,000 rpm for 30 minutes. The sediment was inoculated into selenite or tetrathionate broth.

In methods 1, 4, 5, and 6 both selective and differentiating media were used after the enrichment broth incubation. Standard plate streaking procedures were used on desoxycholate, MacConkey and eosin-methylene blue agar. Suspicious colonies on these plates, as well as on Millipore membranes used with methods 2 and 3, were transplanted to triple sugar iron and urease medium. Growth in tubes showing a glucose positive reaction as well as lactose and urea negative reactions were transplanted to a variety of media to detect motility, indol, hydrogen sulfide, fermentation of mannitol, and growth in citrate. Whenever necessary, salicin, dulcitol, arabinose, inositol, and adonitol as well as lysine-decarboxylase were used for identification of the isolated specimens. Those cultures giving suspicious biochemical

tests for Salmonella or Shigella were then studied serologically using specific antisera.

Enterovirus Detection

Fresh human placentas were obtained from Tucson Medical Center and the amnion was stripped and processed according to standard methods (Merchant, Kahn and Murphy, 1964). The primary human amnion cells were grown in 10% calf serum Melnick A medium with 100 units of penicillin and 0.1 mg per ml of streptomycin in Wallis-Melnick test tubes. One-half gallon water samples were processed according to the method of multiple Millipore membrane filtration and virus concentration developed by Wallis and Melnick (personal communication). This includes the following steps:

1. Clarification of water by filtration through a 142 mm fiberglass prefilter.
2. Removal of bacteria, yeasts, and molds by filtration through a 0.22 $\mu$ 293 mm Millipore membrane.
3. Stabilization of viruses in the water by addition of magnesium chloride to 1 M concentration.
4. Precipitation of non-viral proteins in the water by addition of 2 ml of 1% protamine sulfate, and continous stirring for 30 minutes.

5. Removal of precipitated non-viral proteins by filtration through a 142 mm fiberglass prefilter.

6. Concentration of the viruses by adsorption onto a 0.45 $\mu$ 47 mm Millipore membrane during the final filtration of the sample.

7. Releasing the viruses by grinding the membrane in a mortar and pestle with 2 ml of foetal calf serum and alundum.

The fluid containing the viruses was then removed, diluted with an equal volume of 2% Agamma calf serum, Melnick B medium and frozen at -65 C overnight or until cultured cells were available. Subsequently, 12 tubes of human amnion having a complete monolayer of cells were washed with balanced salt solution, inoculated with 0.25 ml amounts of the thawed sample, followed by the addition of 0.75 ml of 2% agamma calf serum Melnick B medium. Two uninoculated control tubes received one ml of medium alone. Tubes were incubated at 35 C and examined daily for evidence of cytopathogenic effect. Inoculated cultures were compared to the uninoculated controls. Cultures showing cytopathic effect, were frozen at -65 C, thawed, and reinoculated in the same manner to demonstrate that the virus isolate could be passed. Such isolates were frozen at -65 C for later identification of virus type. Negative cultures were incubated for 2 weeks, frozen at -65 C, thawed, and reinoculated into amnion tubes to confirm the absence of virus.

# RESULTS

## Standard Bacterial Counts

Table 1 summarizes the results obtained for the standard count of bacteria, at various sampling sites. The data were obtained from May, 1966 to February, 1967. During this period, chlorination of water at the Tucson Sewage Plant was interrupted in order to complete a program of sewage plant expansion. The average temperature of the water samples during this period was 88 F from May to August and 73 F from December to February.

The lowest count of bacteria observed with raw sewage was $10^6$ organisms per ml which occurred in May while the highest count was $3 \times 10^8$ per ml which occured in July. In general, for raw sewage, the standard bacterial counts were higher during the summer months and fell off in the winter months. As expected, the activated sludge treatment was more effective than the trickling filter system in reducing the number of bacteria. Activated sludge reduced the number of bacteria, 96.1% while the trickling filter only reduced the count 65%, under the conditions of this experiment.

Variable results were obtained in HPI, in some cases a decrease from ASE was noted, while in others an actual increase was evident.

TABLE 1.--Standard Counts of Bacteria at Various Sites During Six Months. Average of Duplicate Plates Times $10^6$ per ml.

| Sample No. | Sewage Plant Influent (SPI) | Activated Sludge Effluent (ASE) | Trickling Filter Effluent (TFE) | Holding Pond Influent (HPI) | Holding Pond Effluent (HPE) |
|---|---|---|---|---|---|
| 1 | 301 | .8 | | | |
| 2 | 20 | | | .001 | .7 |
| 3 | 300 | 10 | 100 | .0004 | .4 |
| 4 | 20 | .1 | 2 | 3 | 50 |
| 5 | 8 | .1 | | .005 | 30 |
| 6 | 50 | .08 | 6 | .0002 | .002 |
| 7 | 40 | .04 | 4 | | 7 |
| 8 | 20 | 1 | 1 | 5 | 1 |
| 9 | 10 | .7 | 7 | 3 | 7 |
| 10 | 6 | .8 | 3 | 3 | 2 |
| 11 | 8 | 2 | 3 | 3 | 3 |
| 12 | 3 | 2 | 5 | 5 | 3 |
| 13 | 8 | .6 | 3 | 5 | 4 |
| 14 | 6 | .6 | 4 | 2 | 3 |
| Mean | 35.7 | 1.4 | 12.5 | 1.7 | 8.2 |
| % Reduction from SPI | | 96.1 | 65 | 95.3 | 77.1 |

However, there was no significant difference in the overall percentage reduction of bacteria from the two sampling sites (96.1 to 95.3%). Similar irregular results were observed when comparing with HPE. In some cases there was a greater number of bacteria observed in HPE than in HPI and in others the opposite was true. The mean percentage reduction of bacteria for the two sites appears to be significantly different (95.3% for HPI to 77.1% for HPE), suggesting some multiplication of the bacteria in the holding pond.

The data obtained for the bacterial counts at the pilot filter influent and effluent sites are presented in Table 2. These results indicate that the pilot filter was quite effective in removing bacteria as evidenced by a bacterial reduction of approximately 99.6%. It should be noted that the mean of the pilot filter influent ($1.1 \times 10^6$) was essentially the same as the mean of activated sludge effluent ($1.4 \times 10^6$). This was expected since ASE was used to load the pilot filter (Fig. 1).

The effect of the time of the year on the standard bacterial counts from various sampling sites is depicted in Figure 2 and 3. For ASE and TFE it is clear that the greatest number of organisms was present during July which coincides with the higher number present in raw sewage at that time of the year (Fig. 2). The holding pond influent and effluent on the other hand showed two peaks, one in July and the second in December (Fig. 3). The significance of this double peak is not clear, but it may be related to the initiation of loading the pilot

TABLE 2. --Standard Counts of Bacteria in Pilot Filter Influent and Effluent. Average of Duplicate Plates Times $10^6$ per ml.

| Sample No. | Pilot Filter Influent (PFI) | Pilot Filter Effluent (PFE) |
|---|---|---|
| 1 | 1 | .03 |
| 2 | .7 | .003 |
| 3 | .8 | .003 |
| 4 | 2 | .0002 |
| 5 | 5 | |
| 6 | .3 | .0007 |
| 7 | .3 | .0007 |
| 8 | .2 | .001 |
| 9 | .2 | .001 |
| 10 | .07 | .0007 |
| Mean | 1.1 | .004 |
| % Reduction from PFI | | 99.6 |

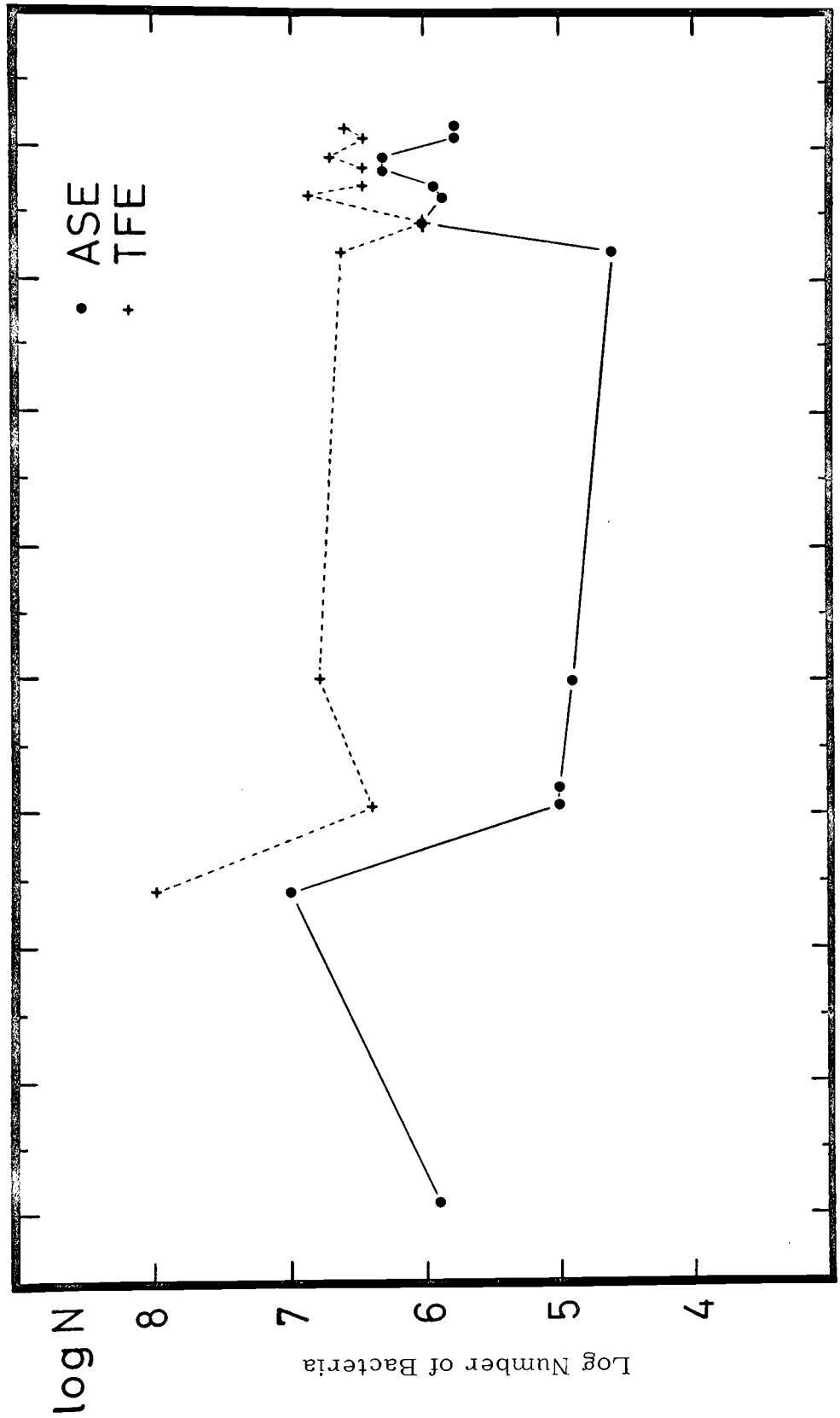



Fig. 2. --Standard Counts of Bacteria of Activated Sludge and Trickling Filter Effluents During Six Months.

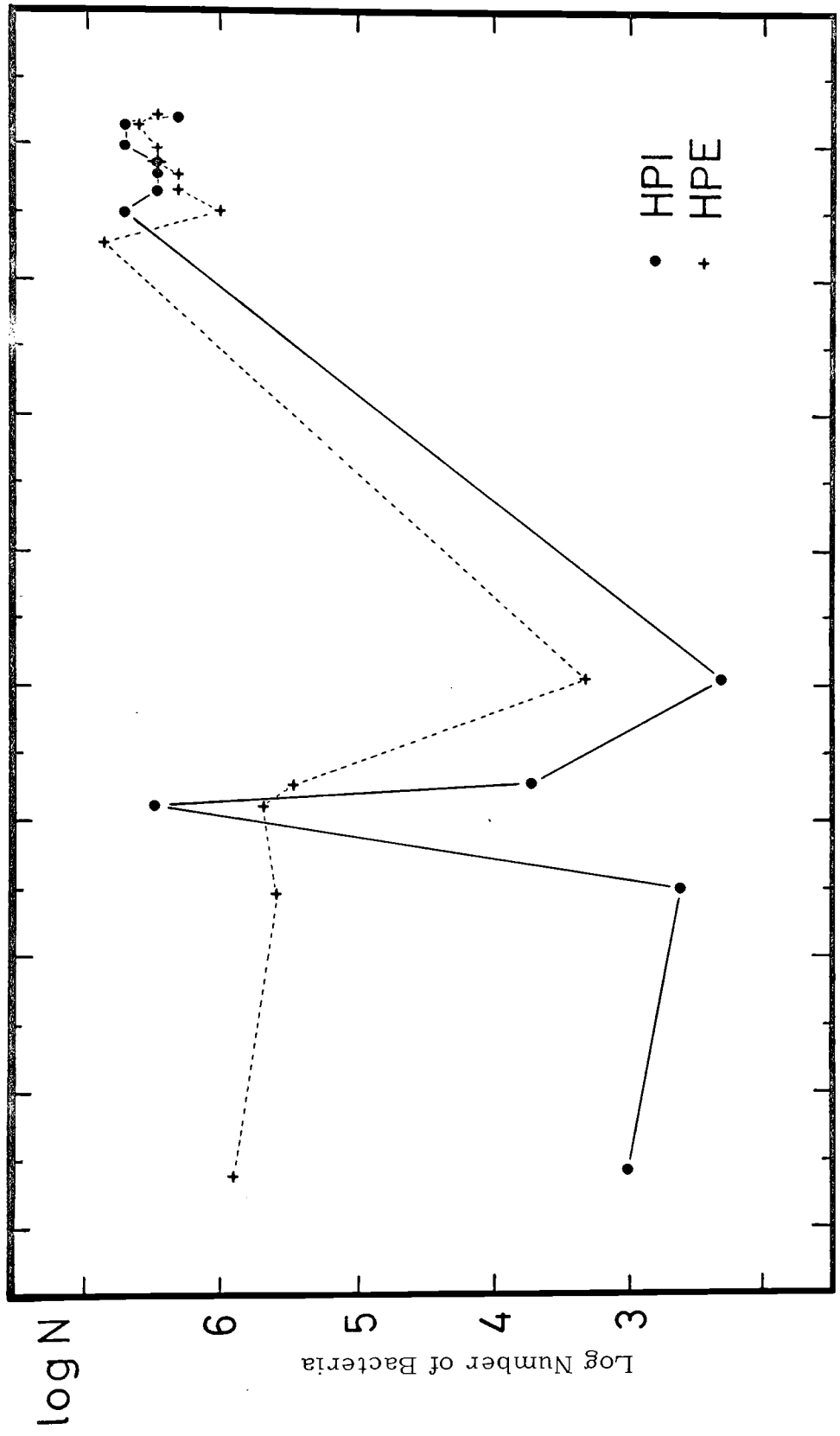



Fig. 3.--Standard Counts of Bacteria of Holding Pond Influent and Effluent During Six Months.

filter with ASE which therefore resulted in an increased quantity of poorer trickling filter effluent reaching the holding pond in an undiluted form (Fig. 1).

A comparison of the variation in standard bacteria counts between December and February for the PFI and PFE is presented in Figure 4. The picture here is complicated in that influent water takes approximately 21 to 25 days before it becomes effluent. Thus, the peak bacterial count for PFI on December 28, 1966 occurs at approximately the lowest bacterial count for PFE but then starts rising one week later. The most interesting feature of PFE was the relatively high bacterial count occurring on December 15, 1966. Although the corresponding PFI bacterial level is not known, the high bacterial count for the PFE correlates well with the date when the enterovirus echo 20 was detected in the PFE (P. Shrivers, personal communication).

Coliform Density

The results of coliform density expressed as Most Probable Number per 100 ml are summarized in Table 3. For any given sample site, fairly constant results were generally observed. Similar to results obtained with standard bacterial count, activated sludge treatment removed more coliforms (88.6%) than trickling filter treatment (39.8%). The coliform results with HPI and HPE were opposite to those observed

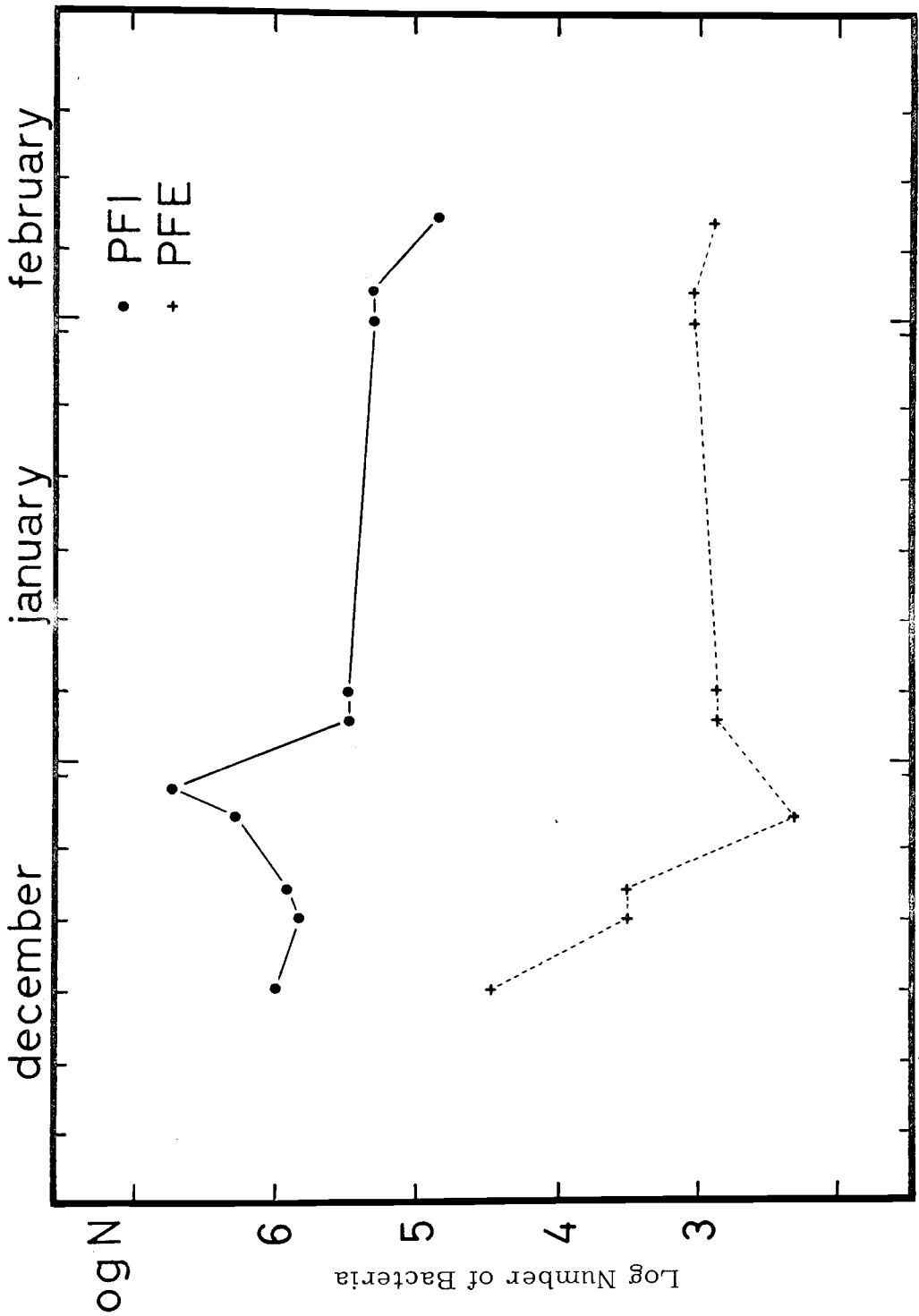



Fig. 4.--Standard Counts of Bacteria of Pilot Filter Influent and Effluent During Three Months.

TABLE 3.--Most Probable Number of Coliforms at Various Treatment Sites During 3 Months. Data Corrected for Bias Times $10^4$ per 100 ml.

| Sample No. | Sewage Plant Influent (SPI) | Activated Sludge Effluent (ASE) | Trickling Filter Effluent (TFE) | Holding Pond Influent (HPI) | Holding Pond Effluent (HPE) |
|---|---|---|---|---|---|
| 1 | 4,900 | 330 | | | 330 |
| 2 | 9,400 | 310 | 1,400 | 480 | 790 |
| 3 | 2,300 | 330 | 2,300 | | 490 |
| 4 | 3,300 | 490 | 490 | 490 | 460 |
| 5 | 1,300 | 490 | 1,100 | 490 | 790 |
| 6 | 4,900 | 1,300 | 1,300 | | |
| 7 | 4,900 | 490 | 2,300 | 1,300 | 790 |
| 8 | 3,300 | 490 | 3,300 | 3,300 | 490 |
| 9 | 1,700 | 110 | 4,900 | 790 | 490 |
| 10 | 7,000 | | 3,300 | 4,600 | 1,100 |
| 11 | 3,300 | 460 | 4,900 | 3,300 | 330 |
| Mean | 4,200 | 480 | 2,530 | 1,843 | 626 |
| % Reduction from SPI | | 88.6 | 39.8 | 56.2 | 85.1 |

for the total bacterial counts. In this case there was an actual decrease in the number of coliforms during the holding period, (56% to 85% reduction).

Coliform density determinations for the pilot filter influent and effluent are presented in Table 4. The data indicates that the pilot filter was extremely effective and reduced the coliform population 99.9%.

## Salmonella and Shigella Determination

A total of 108 samples of water was tested for Salmonella and Shigella by the various methods described. Twenty four out of 108 were taken at SPI, 19 at ASE, 15 at TFE, 15 at HPI, 13 at HPE, 15 at PFI, and 7 at PFE. In spite of the variety of methods used, I was unable to detect any Salmonella or Shigella species. These negative results subsequently led to a study by Mr. Cliff Butler to learn why Salmonella and Shigella could not be detected and a brief explanation for these failures will be presented in the discussion.

## Enterovirus Detection

Seven one-half gallon samples of water were processed by the multiple millipore membrane technique, for enterovirus detection in this work. They included two from SPI, two from ASE, and three from TFE. Considerable difficulty was encountered in the testing procedure because of bacterial contamination within the first 24 hr in inoculated

TABLE 4. --Most Probable Number of Coliforms in Pilot Filter Influent and Effluent During 3 Months. Data Corrected for Bias Times $10^4$ per 100 ml.

| Sample No. | Pilot Filter Influent (PFI) | Pilot Filter Effluent (PFE) |
|---|---|---|
| 1 | 700 | |
| 2 | 49 | .002 |
| 3 | 330 | |
| 4 | 45 | .011 |
| 5 | 79 | .49 |
| 6 | 490 | .017 |
| 7 | 49 | .24 |
| 8 | 330 | |
| 9 | 17 | .17 |
| Mean | 231 | .15 |
| % Reduction from PFI | | 99.9 |

tubes of human amnion. Apparently, Millipore membrane failure due to cracking occurred during the multiple filtration processing and thus, four out of seven processed samples could not be assessed for viruses because of bacterial contamination. Of the three samples successfully processed, virus was detected and passaged from one SPI and one TPE, while the ASE was negative. Eleven out of 12 tubes showed cytopathogenic effect with the SPI sample, while with the TFE specimen, 12 out of 12 tubes showed CPE. Although the findings can only be considered preliminary because of the sample size, they suggest a relatively high concentration of virus in SPI or TFE and a relatively low non-detectable concentration of virus in the one ASE tested.

## DISCUSSION

Certain factors which might influence the data must be taken into consideration. For example, the standard plate count does not count all the bacteria present in a water sample. By this method only those bacteria which grow in the particular medium used here--plate count agar--can be detected and not those nitrifying bacteria, strict anaerobes, etc., which do not grow or grow very slowly in ordinary culture media (Prescott, Winslow, and McCrady, 1946; Favero, Drake, and Randall, 1964). Besides the water temperature, the temperature of incubation affects the results. Under our conditions, the average temperature of the water samples was 11 C cooler during the winter months than during the summer. Hence the concentration of bacteria during the winter was less. The poured plates used for counting were incubated at 35 C. At this temperature only those mesotrophic bacteria develop which are better adapted to the animal body as well as those which live in soils and water. The determination of the number of organisms growing at body temperature is purposely biased so that it may throw light on the presence of direct sewage pollution coming from human or animal excreta (Prescott, Winslow, and McCrady, 1946).

In counting the bacteria, only those plates were chosen which showed 30 to 300 colonies. In this case no significance can be attached

to minor differences between the individual plate counts; it is very common to find large differences. In ordinary sanitation work, differences of ± 25% in results have no important influence on the practical interpretation of the results, since the range between the counts of safe and unsafe waters is enormously greater (Prescott, Winslow, and McCrady, 1946).

The presence of coliform organisms has been used for many years as indicators of water pollution. But in the last few years it has been criticized as an inadequate method to determine the quality of water. Salmonellosis outbreaks from water with low coliform counts (Greenberg and Ongerth, 1966), and the large number of staphylococci and other pathogenic organisms from nose, mouth, and throat in swimming pools (Favero, Drake, and Randall, 1964), have made investigators look for other techniques which might give a more realistic picture of human pollution. The search for enterococci as well as fecal Escherichia coli are examples of the methods proposed in the literature (Clark and Kabler, 1964). ". . . the modern interpretation of detecting coliforms depends on the objectives of the pollution investigation. In some studies, the total coliform group will be the indicator of choice, whereas another investigation with a different objective may better utilize a portion of the group or even a single species" (Clark and Kabler, 1964).

The method chosen to determine the amount of coliforms in this study was the multiple fermentation tube method and coliform density was reported as Most Probable Number (MPN). This method must be considered only as an index of the number of coliform organisms present (Thomas, Woodward, and Kabler, 1956) and as a statistical method, it has many inaccuracies. Recently, a new method, the filter membrane, has been claimed as the best one to determine the amount of coliforms (Clark and Kabler, 1952; Thomas, Woodward and Kabler, 1956; Eliassen, 1957). However, it also has certain disavantages that make the MPN method still useful and one that should be used as frequently as the membrane filter (Shipe and Cameron, 1954; Kabler, 1954; Lieber, 1955; Thomas, Woodward and Kabler, 1956; Clark, Kabler and Geldreich, 1957).

For the purposes of this investigation, it was felt that the standard bacterial count and coliform density determined by MPN method would yield sufficient information to determine what effluent to use to charge the filter. Taking into consideration some of the above mentioned factors which affect results, it is, perhaps, fortunate that treatment of effluents with chlorine was interrupted during the course of these experiments for it permitted a better evaluation of the biological treatment. This is shown by the data from sampling point HPI or PFI where, in spite of the non-chlorination of the water, there was not a significant difference between the numbers of bacteria at these points

and with the ASE. Those cases where the amount was increased, can be attributed to exposure of the water in a lengthy open channel in order to reach the holding pond. As expected, activated sludge treatment was considerably better than trickling filter treatment.

With respect to the variation in results observed from HPE, I attribute these to 1) differences in water temperature at the times of sampling (Prescott, Winslow and McCrady, 1946), 2) the time of day when samples were taken as influenced by sun light (McKinney, 1962), and 3) the level of the water which on certain days was not even overflowing into the river. Previous studies by other workers have demonstrated that there is a wide variation in the bacterial density from the surface to the bottom of the pond (Malina and Yousef, 1964). So too, the overall higher numbers of HPE to HPI can be explained on the basis of bacterial multiplication, but not of the coliform variety.

The results obtained from PFI and PFE demonstrate clearly that the pilot filter effectively removed 99.6% of the total bacteria and 99.9% of the coliforms. These results are in agreement with those of Phillips and Mees (personal communication).

The failure to find Salmonella and Shigella in the raw sewage of the Tucson Sewage Treatment Plant, cannot be attributed to absence of these organisms in wastewater. There are many examples in the literature in which investigators have been successful, and there are others where workers were unable to isolate Salmonella or Shigella from

sewage. Many factors might influence the isolation of these bacteria from wastewater. The initial population in raw sewage, and the competition for nutrients by other living organisms as well as overgrowth by less fastidious bacteria were considered important factors by McKenny, Langley, and Tomlinson (1958). The presence of antibiotic substances isolated from Bacillus subtilis that were active against Shigella flexneri, Salmonella typhosa and S. paratyphi was suggested by Weaver and Boiter (1951). Wang, Dunlop, and De Boer (1956), over years of study were unable to isolate Shigella from wastewater and irrigation water samples and attributed this failure to a lack of necessary cations in the samples plated on media with bile salts. The same investigators found that survival of Sh. flexneri in fresh wastewater and irrigation waters is independent of the number of phage particles present, but depends on the number of total bacteria and the temperature of the water. Guelin and Gozdowa (1952) reported that enterobacteria in polluted waters decreased progressively in the first few days and then dropped abruptly on the seventh day, accompanied by a rapid clearing of the medium. No clearing was observed in autoclaved waters.

My negative results subsequently led to a study by Mr. Cliff Butler to learn why Salmonella and Shigella could not be detected in sewage from the Tucson Plant. Data by Butler (personal communication) indicate that the high concentration of indigenous organisms cause

antagonistic effect against S. typhimurium and rapidly decrease the probability of recovery.

Although the main purpose of this work was not the detection of viruses, the finding of viruses in two samples, one from SPI and one from TFE, indicates the important role that wastewater may play in the transmission of infectious diseases.

So far as the major aim of this project is concerned, based on coliform and standard bacterial counts, I can say that activated sludge treatment yields the best quality water and it should be used first to charge the pilot filter. Activated sludge effluent has always demonstrated its efficiency and my results are in agreement with others (Askew et al., 1965).

As McKinney (1965) stated: "Activated sludge poses a definite challenge for research and development in the future. With the problems of increasing water pollution, new modifications of activated sludge will be needed as fast as they can be developed."

If a second source of effluent is needed, I would recommend HPE for it is better quality water than TFE which is the poorest choice of all.

## LIST OF REFERENCES


APHA, AWWA, and WPCF, 1960. Standard Methods for the Examination of Water and Wastewater. 11th edition. APHA Inc., N.Y.

Arnold, C., Hedger, H., and Rawn, A., in McGauhey, P. 1957. The Why and How of Sewage Effluent Reclamation. Water and Sewage Works. 104:265.

Askew, J. B., Bott, R., Leach, R., and England, B. 1965. Microbiology of Reclaimed Water from Sewage for Recreational Use. American Journal of Public Health. 55(3)453.

Bloom, H., Mack, W. Mallmann, W. 1958. Enteric Viruses and Salmonellae Isolation. II. Media comparison for Salmonellae. Sewage and Industrial Wastes, 30: 1455.

Burrows, W. 1963. Text-book of Microbiology. 18th ed. W. B. Saunders Co.

Clark, H. F., Kabler, P. W., and Geldreich, E. 1957. Advantages and Limitations of the Membrane Filter Procedure. Water and Sewage Works. 104:385.

Clark, H., and Kabler, P. 1964. Reevaluation of the Significance of the Coliform Bacteria. Journal of American Water Works Association. 56:931.

Collett, E., Johnston, R., Ey, L., and Croft, E. H. 1953. Isolation of Salmonella typhosa from Well Water by Membrane Filter Technique. American Journal of Public Health. 43:1438.

Chang, S., Stevenson, R., Bryant, A., Woodward, R., and Kabler, P. 1958. Removal of Coxsackie and Bacterial Viruses in Water by Flocculation. American Journal of Public Health. 48:51.

Dunlop, S. G., Twedt, R. M., and Wang, W. L. 1951. Salmonella in Irrigation Water. Sewage and Industrial Wastes. 23:1118.

Dunlop, S. G., Twedt, R. M., and Wang, W. L. 1952. Quantitative Estimation of Salmonella in Irrigation Water. Sewage and Industrial Wastes. 24:1015.

Eckenfelder, W., and O'Connor, D. 1964. Biological Waste Treatment. Fermagon Press.

Eliassen, R. 1957. Appraisal of Membrane Filters. Water and Sewage Works. 104:103.

Favero, M. S., Drake, C. H., and Randall, G. 1964. Use of Staphylococci as Indicators of Swimming Pool Pollution. Public Health Reports. 79:61.

Guelin, A., Gozdowa-LeBois. 1952. Ann. Inst. Pasteur. 82:78

Greenberg, A. E., and Ongerth, H. 1966. Salmonellosis in Riverside, Cal. Journal of American Water Works Association. 58:1145.

Heukelekian, H. 1953. Microbiology of Water and Sewage. Annual Review of Microbiology. 7:461.

Heukelekian, H., and Albanese, M. 1955. Enumeration and Survival of Human Tubercle Bacilli in Polluted Waters. Sewage and Industrial Wastes. 28:1094.

Jensen, E. 1954. Presence and Destruction of Tubercle Bacilli in Sewage. Bull. World Health Organization. 10:171.

Kabler, P. and Clark, H. 1952. The Use of Differential Media with the Membrane Filter. Journal of American Public Health. 42:390.

Kabler, P. 1954. Water Examinations by Membrane Filter and Most Probable Number Procedure. American Journal of Public Health. 44:379.

_____. 1959. Removal of Pathogenic Microorganisms by Sewage Treatment. Sewage Industrial. 31:1373.

Kelly, S. M., Clarck, M., and Coleman, M. 1955. Demonstration of Infectious Agents in Sewage. American Journal of Public Health. 45:1438.

Lieber, M. 1955. A Critique on The Membrane Filter. Water and Sewage Works. 102:400.

Mack, W. N., Mallmann, W. L., Bloom, H. H., and Krueger, B.J. 1958. Isolation of Enteric Viruses and Salmonella from Sewage. Sewage and Industrial Wastes. 30:957.

Malina, J. F., and Yousef, Y. A. 1964. The Fate of Coliform Organisms in Waste Stabilization Ponds. Journal of Water Pollution Control Federation. 36:1432.

McGauhey, P. H. 1957. The Why and How of Sewage Effluent Reclamation. Water and Sewage Works. 104:265.

McKinney, R. E., Langley, H. E., and Tomlinson, H. D. 1958. Survival of Salmonella typhosa During Anaerobic Digestion. I. Exposition Methods and High-Rate Digester Studies. Sewage and Industrial Wastes. 30:1469.

McKinney, R. E. 1962. Microbiology for Sanitary Engineers. McGraw Hill.

_____. 1965. Research and Current Developments in the Activated Sludge Process. Journal of Water Pollution Control Federation. 37:1696.

Merchant, E., Kahn, R., and Murphy, W. 1964. Handbook of Cell and Organ Culture. Burgess Publishers Co.

Pelczar, M., and Reid, R. 1965. Microbiology. 2nd ed. McGraw Hill.

Prescott, S., Winslow, C. H., and McCrady, M. 1946. Water Bacteriology. John Wiley and Sons.

Ringen, L., and Drake, C. H. 1952. A study of the Incidence of Pseudomonas aeruginosa from Various Sources. Journal of Bacteriology. 64:841.

Shipe, E. L. and Cameron, G. M. 1954. A Comparison of the MF with the MPN for Coliform Densities from Several Waters. Applied Microbiology. 2:85.

Thomas, H., Woodward, R., and Kabler, P. 1956. Use of Molecular Filter Membrane for Water Potability Control. Journal of American Water Works Association. 48:1391.

Wang, W. L., Dunlop, S. G. and De Boer, R. G. 1956. The Survival of Shigella in Sewage. Applied Microbiology. 4:34.

Weaver, R. H. and Boiter, T. 1951. Trans. Kentucky Academy of Science. 13:183, in Heukelekian, H. 1953. Microbiology of Water and Sewage. Annual Review of Microbiology 7:461.