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AIRBORNE BACTERIA FROM AN ACTIVATED SLUDGE PLANT

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CHAPTER I

INTRODUCTION

It is estimated that the total world population at the time of Christ was about 250 million. Today, the population numbers over 3.5 billion. Should the population increase continue at the present rate of 1.75 per cent compounded annually, society has every reason to suppose that, by the year 2000, there will be 7 billion people on the Earth. Of these, 90 per cent will reside in urbanized areas comprising 10 per cent of the total land space available (1).

It has long been recognized that crowding increases the contact rate of infectious diseases (2). Respiratory diseases are of particular concern in crowded areas, yet approximately 70 per cent of all respiratory infections are of unknown etiology. Areas of different bacterial concentrations exist above a city as was shown by Mill, Robertson and Walker (3) in studies conducted over Oklahoma City in 1970. They observed that areas of greatest bacterial concentration existed over sewage treatment plants and highway systems.

With increasing populations, available space for such essential processes as waste disposal decreases. Potentially desirable methods of waste disposal are those which require relatively little space, can process large volumes of sewage in a short time, and produce no offensive odors in the normal operation of the plant. The latter is preferable because of the presence or projected proximity of human dwelling areas.

One method of sewage disposal that has found acceptance is the activated sludge process in which large volumes of compressed air are forced through sewage in a tank. Aerobic conditions are therefore maintained. The presence of various aerobic microscopic life forms capable of oxidizing organic matter effect rapid clarification, enzymatic decomposition, oxidation, and a decrease in bacterial content, usually within 5 to 8 hours. In 1962 Barth (4) reported that 60 per cent of the population of the United States provided some type of disposal treatment of its waste water, and that 32 per cent of that waste was treated by the activated sludge method.

Human excreta averages about 83 grams of feces and 970 grams of urine per day per person (5). With a projected world population of 7 billion people within 29 years, there will be 233,600,000 tons of excreta to dispose of each year. With tonnage of this magnitude, the problem ceases to be one of academic interest, and becomes one of major concern to the field of Environmental Health.

In the activated sludge process, which utilizes compressed air pumped through sewage, there exists the inherent possibility of airborne dispersion of bacteria through the mechanism of droplet nuclei evaporation. These droplet nuclei are small droplets, which have so little moisture content that the water evaporates rapidly, leaving very light particles like bacteria suspended in the air for an indefinite time. These particles may be carried by air currents some distance from their source (2). The dispersal of such contaminants in the atmosphere does not remove the material; they are simply diluted by increased volumes of air.

At one time in Man's history, it was assumed that the atmosphere was an important vehicle of infection, and disease was thought to be due to "Miasmas" floating about, especially on damp or foggy night air. By substituting "Malaria carrying mosquitoes" for "Miasmas", the ancient fear of damp night air does not seem so far afield as might be supposed upon first inspection, and further studies of possible mechanisms of disease dispersion through the atmosphere may prove profitable.

CHAPTER II

LITERATURE REVIEW

Human excreta will at times contain organisms of disease, and sewage containing discharges of many persons can be expected to contain a few pathogens. The most important of the waterborne diseases are those of the intestinal tract. Of these, typhoid fever, paratyphoid, bacillary dysentery, cholera and infectious hepatitis are known to be transported in sewage (1).

Common sewage bacteria from soil or feces are the coliform group, fecal streptococci, clostridia, micrococcus, bacteriodes, cytophages, pseudomonads, achromobacters, alcaligenes, bacilli, lactic bacilli and spirochetes (6). The most important protozoans found in sewage throughout all seasons of the year are Verticella, Opercularia, Epistylis and Chilodonella. Summer organisms are as follows: Stylonychia, Oxytricha, Lionotus, Cychidium, Paramecium, Cinetochilum, Loxodea, Trichopelma, Metopus, Podophrya, Habrotrocha, Encemtrum, Chlorella, Scenedismus and Stigeoclenium. In winter, the predominance of some naviculoid diatomes has been observed (7). At least 60 types of enteric viruses have been

isolated from sewage. These viruses usually appear in the final effluent, even after normal chlorination. By improving the methods of isolation of viruses, the presence of enteric viruses was demonstrated in sewage throughout the year by Lund, Hedstron and Jantzen (8).

Studies of the mechanisms of microorganism removal from sewage in activated sludge tanks have been performed. In a study conducted by Kelly, Sanderson and Neidl (9), removal of poliovirus and bacteriophage by activated sludge was shown to occur in at least two steps: trapping viruses in the capsular material of sludge floc and its nutrient, and the mechanical settling of floc. The isolation of at least four strains of bacteria with antiviral activity suggested that biological antagonism was a third step. Recent research carried out on antibiotic-forming strains of fungi contained in sewage indicated that bacteria are reduced in number by their presence. Antibiotic forming strains were not increased in sewage samples in which mesophilic bacteria were reduced (10). Clark et al. (11) established that an average coliform removal of 97 per cent was not unusual in activated sludge plants.

Experiments conducted by Hartman and Laubenberger (12) in 1968 related to the influence of turbulence on the vigor of activated sludge floc showed that at high concentrations of organic matter, transport of oxygen to the cell surface was the rate-limiting link in biodegradation of organic

material. An increase in oxygen pressure resulted in an increase in oxygen consumption. Turbulence created by the inflow of compressed air served to break apart agglomerated floc into smaller floc within the activated sludge tank. The finer particles promoted exposure of contact area between bacteria and sewage: a phenomena which resulted in greater oxygen uptake and more rapid oxidation of waste. Large liquid droplets that occur do not necessarily fall directly to a horizontal surface, but evaporate to a residue known as droplet nuclei. A 100 μ droplet of pure water in dry air evaporates completely in 2 seconds, which is one-third of the time required for it to fall approximately 6 ft in still air (13). Evaporation is slower if the droplet contains proteinaceous material (14).

Cox (15) found that the survival pattern of Escherichia coli sprayed from distilled water into an atmosphere of nitrogen showed marked instability in regions of high relative humidity, and better stability in regions of low relative humidity. He was able to determine that the loss of viability was not caused by DNA inactivity, DNA synthesis inhibition or inhibition of cell wall division, but rather by failure of RNA synthesis, protein synthesis or energy production. Hess (16) found that, while the colony-forming ability of aerosolized Serratia marcescens was rapidly destroyed by contact with 0.25 per cent or more oxygen, the same organisms in a completely hydrolized state were insensitive to

oxygen at pressures up to 100 pounds per square inch for exposure periods of 4 hours or more. No loss of viability occurred in aerosols of washed cells in air at 97 per cent relative humidity. He proposed that dehydration of the aerosolized cells results in sensitization to lethal effects of oxygen.

Vlodavets (17) found the drop concentration of viable bacteria in air to be a process which depends on the death of bacteria, as well as their sedimentation. The microorganisms Staphylococcus aureus and Sarcina lutea which are adapted to the conditions of the air medium were well preserved at relative humidities between 12 and 98 per cent within the temperature range of 18.5 to 21° C. Their removal from the air was largely through sedimentation. Escherichia coli and Serratia marcescens are Gram negative organisms not adapted to an air medium, and proved much less stable in aerosols. Both organisms died rapidly in relative humidities below 50 per cent, but survived at relative humidities of 70 per cent and above.

Microorganisms of various types have been reported by Proctor (18) at heights ranging up to 20,000 ft. Rogers and Meier (19) reported microorganisms at altitudes up to 36,000 ft.

Napolitano and Rose (20), using the Andersen seive sampler, compared coliform emissions from an activated sludge plant to that of a trickling filter plant. Viable coliform

recovery from the activated sludge plant was roughly ten times greater than the recovery from the trickling filter plant with mechanical turbulence in the activated sludge plant being cited as the probable cause of increased emissions. Ledbetter (21) conducted a series of tests for airborne bacteria on the premises of an activated sludge plant and by sampling at various locations within the plant area, determined that the highest concentration of airborne bacteria occurred downwind from the aeration units. Ladd (22) compared several different methods of sampling sewage treatment plants for bacterial emissions and concluded that the Andersen drum sampler was the sampler of choice for studies of this nature. Ladd also introduced a tracer bacteria, Bacillus subtilis var. globgii into the sewerage system and successfully recovered the organism from the ambient air at the preaeration tank sampling site.

For some of the bacterial and viral diseases as well as many allergic conditions, the evidence that the air is a carrier of the etiological agent is incontrovertible. Anthrax, measles, chicken-pox, Q fever, psittocosis and septic infections are examples of bacterial diseases due to direct contact or dispersion of microorganisms through the air. Fungal diseases such as actinomycosis, histoplasmosis, coccidioidomycosis and blastomycosis are diseases transmitted to man through air dispersion of spores (23). Infection through droplet discharges is important in relation to

respiratory infections. The possibility that bacteria transported through air play a part in alimentary infections is suggested by the findings of a specific serotype of Escherichia coli in the air in cases of gastroenteritis, and of Salmonella derby in dust during an outbreak of infection caused by that organism (2).

The World Health Organization defines health as, "A state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (1). Basically, air pollution is the presence of foreign substances in the air. An air pollution problem arises when the concentration of these substances interfere with the well being of people. A typical legal definition of air pollution is:

> "The presence in the outdoor atmosphere of substances or contaminants, put there by man, in quantities or concentrations and of a duration as to cause any discomfort to a substantial number of inhabitants of a district or which are injurious to public health, or to human, plant or animal life or property or which interfere with the reasonable, comfortable enjoyment of life and property throughout the state or throughout such territories or areas of the state as shall be affected thereby." (24).

A man utilizes about 30 lbs or air daily, compared to 4.5 lbs of water and 2.75 lbs of food. He may refuse suspected water or food but not polluted air. Man can live weeks without food and days without water, but only minutes without air (25). May, Druett and Packman (26) conclude that there is an Open Air Factor which influences the death

rate of organisms exposed to ambient conditions, and suggest that death rates measured in enclosed laboratory air can be highly misleading when projected to the many variables encountered in ambient air. Perhaps more in-the-field studies will serve to overcome those variables in the study of pollution.

CHAPTER III

PURPOSE AND SCOPE

The literature seems to indicate that extensive work has been done concerning the organisms found in activated sludge. Organisms found in ambient air have been identified, and probable explanations for their ability to remain viable in a foreign environment have been explored. Numerous sources of bacteria in ambient air, such as dust, ocean spray and other natural forces have been identified.

The purpose of this investigation was to establish a connection between water pollution and air pollution through the aereation process utilized in an activated sludge plant. In the process of supplying sufficient oxygen for the aerobic biodegradation of wastes, air is blown through sewage-laden water which disperses bubbles and droplets of bacterially contaminated sewage into the ambient air of man.

Specifically, it was the purpose of this study to: (a) identify sufficient bacterial components of one activated sludge plant so that organisms collected from the ambient air downwind from that plant could be traced to the plant as the probable source, (b) to introduce a tracer organism into the

plant system to determine its retention time in the plant and its dispersion parameters in ambient air and (c) to establish modified dispersion patterns utilizing arithmetic - normal graphs.

CHAPTER IV

MATERIALS AND METHODS

The Northside Sewage Treatment Plant, a conventional activated sludge process located in the Lincoln Park area near the zoo in eastern Oklahoma City, was selected for this investigation (Figure 1). At the time of this study the plant was enclosed in a fenced area 495 ft wide by 1309 ft long and was contained in a tree-fringed man-made crater which encompassed the functional areas of the plant. The plant was processing domestic sewage from the Deep Fork Relief Sanitary Sewer at an average rate of 10.8 mgd.

The aereation area consisted of a gallery of eight tanks, each measuring 30 ft wide, 15 ft deep and 140 ft long. Six central tanks of the gallery were in operation during the study period. Each tank had an operational capacity of 473,500 gal. Sewage retention time in the interconnected tanks averaged 4.8 hours, with a 33.3 per cent recycling of sludge. An average of 12 million ft^3 of air per day, or 0.5 million ft^3 of air an hour provided both aeration and agitation of the mixed liquor.

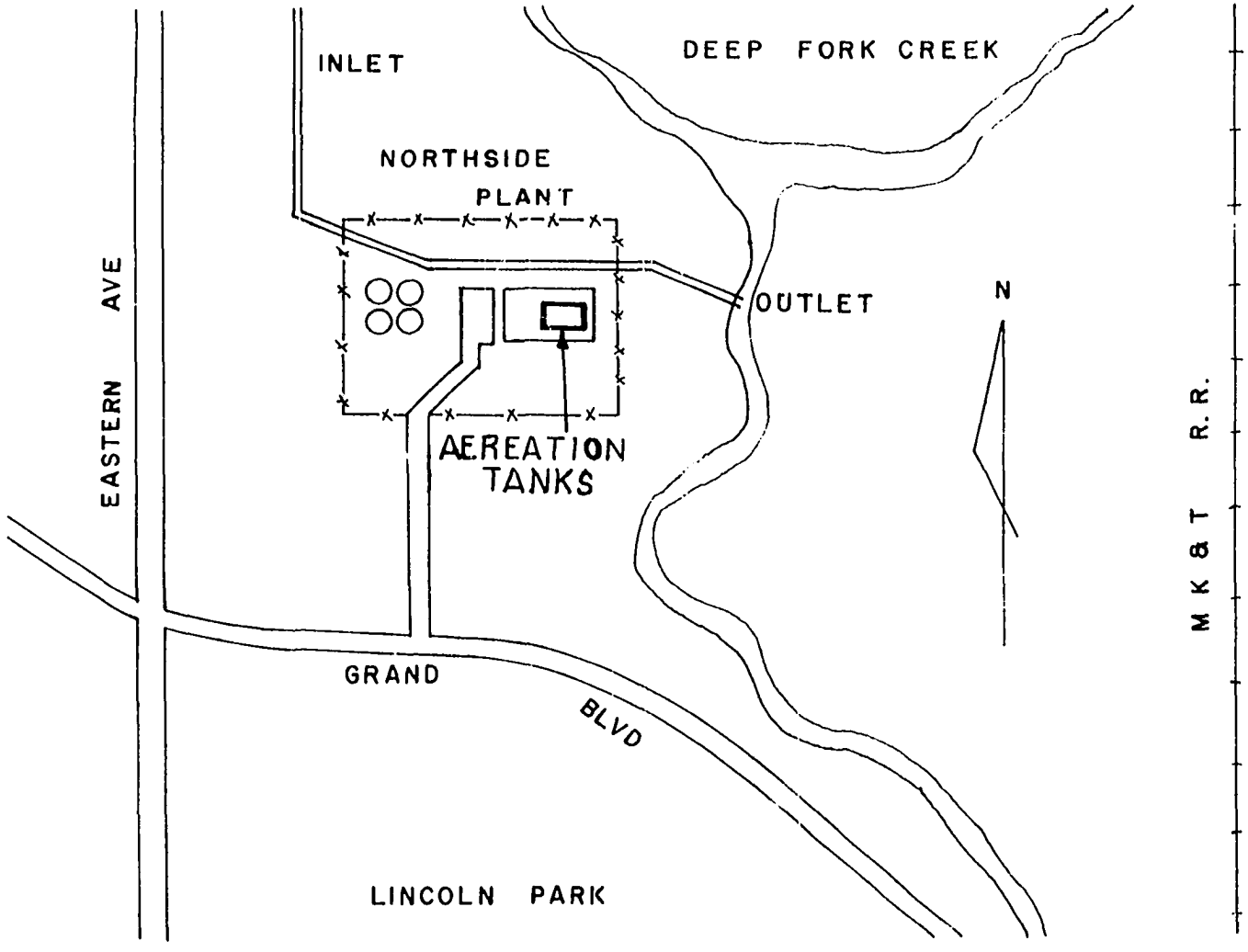

Figure 1. Layout of Oklahoma City Northside Sewage Treatment Plant

Four clarifiers were used to process the effluent from the aereation tanks before discharging into Deep Fork Creek. Waste sludge from the tanks and raw sludge from the primary settling tanks were pumped to two primary digesters backed by two secondary digesters. The digested sludge was pumped to drying beds within the plant. Drainage from the drying beds was recycled into the plant system.

During the study period, an attempt to increase the plant capacity was underway. Floating electrically driven mechanical surface aereators were being placed at different locations in the tanks in order to establish the most economical and functional application of these units in conjunction with the compressed air routinely utilized in the plant. It was proposed that 12 floating aereators would increase the plant capacity to 150 per cent of it's previous capability. Since a central reference point of origin was more amenable to graphic representation, a projected point source, south of the aereation tanks, was used instead of a line source of origin.

Because of a predominant south wind, a site north of the aereation tanks was selected as the sampling area. This sampling area was confined within a 21^{o} angle which extended from the reference point through the northern edges of the aereation gallery. The sampling area was gridded by extending concentric arcs, spaced 20 ft apart, from the north side of the aereation gallery to the plant fence. Radial

lines intersecting the arcs divided the sampling area into 20-ft^2 units. Sampling points were located at the corners of these units, and marked by colored stakes. The arcs were labeled alphabetically beginning at the first arc north of the aereation gallery; the radial lines were labeled numerically beginning at the left side of the sampling area (Figure 2).

The sampling devices used were Andersen Drum Samplers (27). These devices collect airborne bacteria through a limiting orifice by impacting them on an agar coated rotating drum with a surface area of 62 in^2. The drum is contained within a sealed canister; access to the outside atmosphere is through air drawn into the canister through the limiting orifice. The drums moved downward 0.125-inch per revolution, creating a maximum of 27 line deposits per drum. Flow rates were controlled by limiting orifices rated at 1 cfm. Each drum sampler was matched and labelled with it's specific Andersen vacuum pump, then calibrated against a wet flow meter in the laboratory. Each sampler and pump combination was recalibrated and the flow rates confirmed at random intervals throughout the study period.

During each sampling period, temperature and relative humidity were obtained utilizing a Sufft hygrometer, wind speed and wind direction were recorded by a Wong EcoWind 111 wind system, and the barometric pressure was determined by using an Airguide marine barometer. All meteorological data

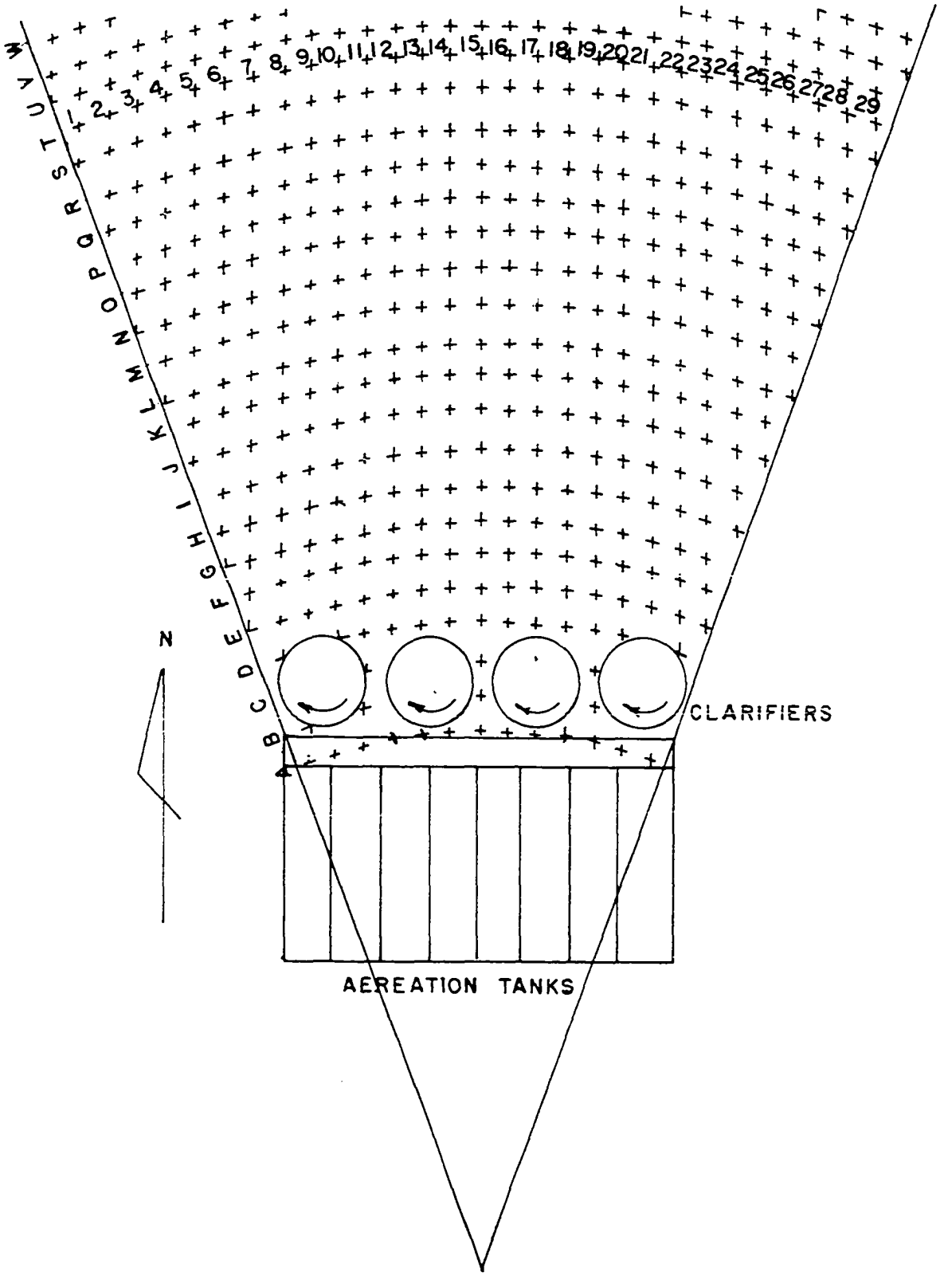

Figure 2. Sampling area ajacent to the aereation tanks

were obtained within the gridded area of the plant at the time of sampling. These data were used in establishing dispersion patterns at the plant.

During each of the 1-hour sampling periods, three air samples were taken. One sample, designated as the control, was taken up-wind from the aereation gallery and was presumed to be representative of the normal ambient air flora. Two were taken in the down-wind sampling area. One, designated as the "wind sample", was located on the grid by the direction of the predominating wind. The other, designated the "random number sample", was located on the same arc of the grid by a table of random numbers of sufficient size to include all possible sampling points on that arc.

Although Prakasam and Dondero (28) stated that increased viable counts were obtained by utilizing agar-solidified sludge liquor in bacterial counts of activated sludge, they found that reproducibility varied and were unable to establish a formulation for their media. Therefore, it was decided to utilize a formulated media with good reproducibility as suggested by the U. S. Public Health Service (29). The media used to coat the drums of the Andersen Drum Samplers was Bacto Nutrient Agar, rehydrated according to the instructions of the manufacturer (30). Wet sponges were kept in the drum canisters during sampling and incubation periods to retard dehydration of the media.

After each sampling period, the Andersen canisters

containing the drums were returned to the laboratory where they were placed in a walk-in incubator pre-set at 35° C. Although optimum growth requirements varied for the organisms collected, this study was concerned primarily with aerobic mesophilic bacteria dispersed from the plant. Aerobic conditions in this study were utilized because: (a) of their application to the sewage plant process, (b) the air borne route of transmission under investigation, (c) and their relevance to possible human infection.

Preliminary incubation periods which exceeded 24 hours resulted in colonies which spread together and lost their identity; therefore, colony counts and isolation procedures were iniatiated after 18 to 20 hours incubation. Colony counts were an enumeration of all surface colonies on the drum agar which were visible to the unaided eye in a well lighted room. Colony counts were recorded in terms of viable bacterial units per ft^3 of air per hour. Isolation procedures consisted of streaking an individual colony from the drum agar to a separate petri dish containing nutrient agar. The petri dish was incubated at 35° C for 24 hours, and an isolated colony from the agar surface was used to inoculate identification media.

The media used for identification were Difco — dehydrated agars and broths. Urea broth and the carbohydrates: xylose, sucrose, arabinose and salicin were purchased in concentrated sterile liquids in order to eliminate possible

decomposition at elevated temperatures. Appropriate amounts of the concentrates were aseptically transferred to presterilized liquid diluents, according to the manufacturers instructions, prior to use.

All inoculated biochemical media were incubated at 35° C. Gram stain and catalase production tests were performed after 24 hours incubation. Methyl red, Voges-Proskauer, nitrate reduction, oxidase and indol production tests were performed after 48 hours incubation. Carbohydrate fermentation tests were incubated for a period of 10 days, unless they became positive earlier, before they were interpreted as being negative. Decarboxylase tests (arginine, lysine and ornithine) were recorded as being negative or positive after 3 days incubation. Sterile petrolatum for anaerobic seals of identification media was obtained by dry oven sterilization at 350° F for a period of 4 hours.

Hucker's modification of the gram stain (31) was employed to stain smears of isolated colonies. The identification scheme of Cowan and Steel (32) was used to place an isolate into a specific Genus, although the terminology of Bailey and Scott (31) was applied where differences between English and American preference occurred. Bergey's manual (33) was consulted in the identification of some species.

Since upwind and downwind samples were taken at the same time using identical equipment under identical

meteorological conditions, statistical analysis of data collected during this study was confined to the paired 't' test. Three series of paired 't' tests were calculated according to the method of Steel and Torrie (34); the first series compared the upwind control colony counts with the downwind sample colony counts; the second series compared the upwind colony counts with the random number sample colony counts; and the third series compared the downwind sample colony counts with the random number colony counts. All tests used the one-tailed 't' test with a level of significance of 0.05.

The tracer organism used in this study was a strain of Serratia marcescens obtained from the Department of Botany and Microbiology of the University of Oklahoma. This culture had been used for several years for teaching purposes, and was considered to be non pathogenic (35). Laboratory studies on the survival rate of the organism in sewage from the aeration tanks of the Northside Sewage Treatment plant were conducted to assure that no extended upset in the active bacterial components of the sludge would occur. A sewage sample of 200 ml was seeded with a 1×10^{9} concentration of S. marcescens grown on a nutrient agar plate for 48 hours at room temperature. Each day, serial dilutions through 10^{-8} were made of the sewage and 0.1 ml transferred to the surfaces of nutrient agar plates. A sterile L-shaped glass spreader was used to distribute

the inoculum evenly over the surface of the media. The plates were incubated at room temperature for 48 hours before the deep red colonies of S. marcescens were counted. The S. marcescens colony count was multiplied by the dilution factor and a survival curve was constructed plotting time in days against the number organisms present per ml of sewage (Figure 3).

Sufficient quantities of the tracer organism for this study were grown in aerated 1-gal glass jugs containing nutrient broth. Five jugs were thoroughly washed in an automatic glassware washer, rinsed in distilled water, and dried. Each jug received 3 l of rehydrated nutrient broth and a two holed No. 6 rubber stopper fitted with one long glass tube which extended well below the surface of the broth and one short glass tube which ended well above the surface of the broth. These broth culture units and 4 ft of rubber hose with the same inside diameter as the glass tubing were sterilized in an autoclave for 15 min at 121° C. The culture units were allowed to cool overnight inside the autoclave and connected in series with 6 inch strips of the sterile hose as they were removed from the autoclave the next morning. The compressed air outlet in the laboratory was utilized as the aeration source. This air passed through a packed column of sterile cotton before it reached the first culture unit. After bubbling through the last culture unit, the compressed air passed through 50 ml of 10^{-3} aqueous

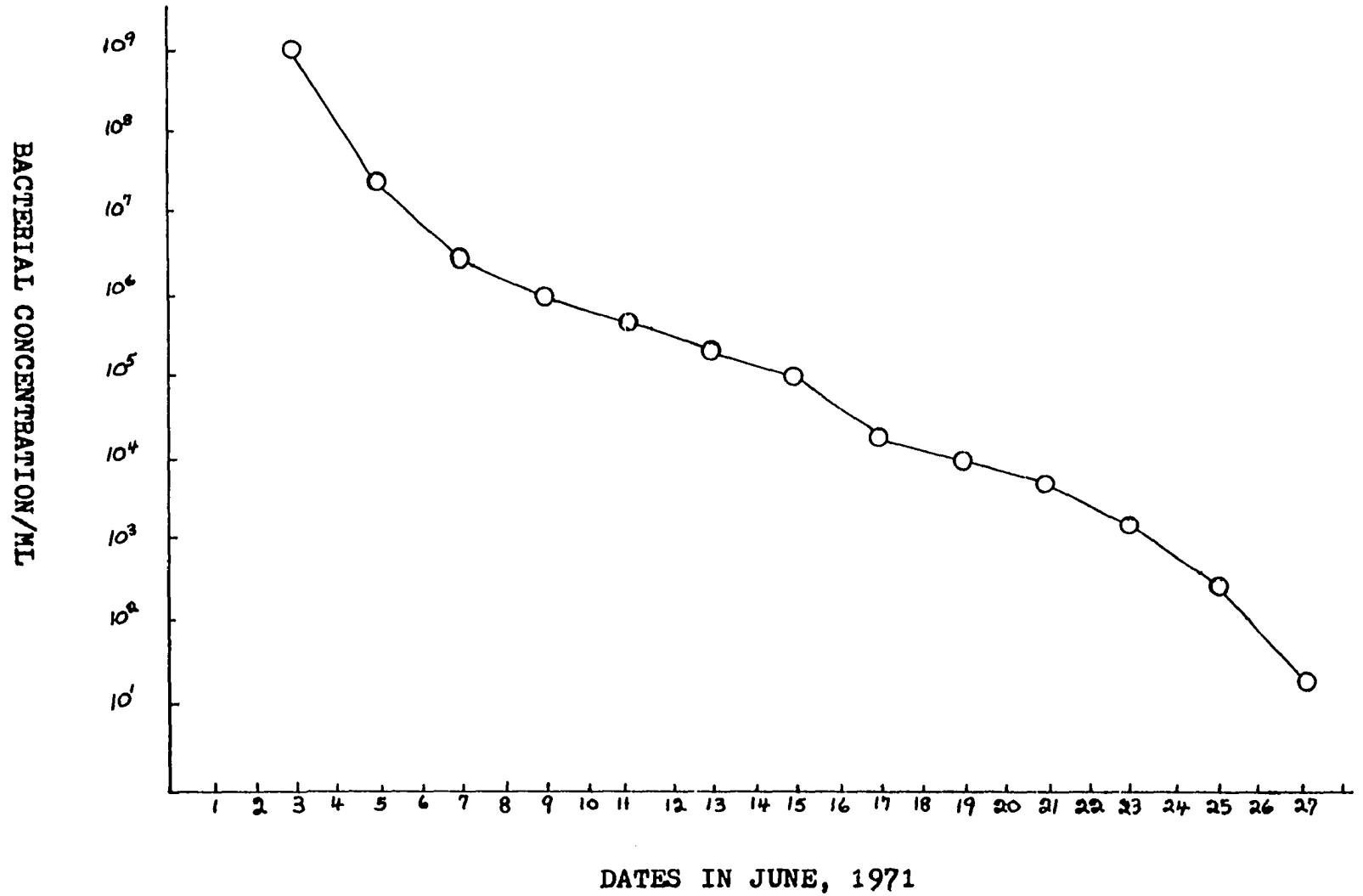

Figure 3. Survival curve of Serratia marcescens in sewage.

mercuric chloride before being discharged into the ambient air. The seeded culture units were aerated for 48 hours at room temperature before being disconnected and sealed as individual units with sterile screw caps. The units were refrigerated to retard further growth, and 1-ml samples of the resuspended cultures were taken from the culture units for bacterial enumeration. Serial dilutions up to 10^{-11} were made, and 0.1 ml quantities of the diluted cultures were spread over the nutrient agar surfaces of petri dishes with a sterile glass rod. These petri dishes were incubated at room temperature for 48 hours before colony counts were performed. The mean of the five colony counts was calculated as the bacterial count per ml of nutrient broth and used in calculating the total number of S. marcescens to be introduced into the sewage treatment plant system.

The organisms were introduced into the plant at the sludge return duct just above the aereation tanks. Previous studies of dispersion throughout the aeration tanks were conducted utilizing the flourescent dye, sodium flourescene. The dye took approximately 3 hours to disperse throughout all six aeration tanks, therefore aerial sampling for S. marcescens began 3 hours after the organism were introduced into the plant system.

Aerial sampling for the tracer organism was accomplished by placing two Andersen Drum Samplers downwind. One was placed on a selected arc sampling point as dictated by the wind

direction, and the other was placed on the same arc with the sampling point selected by a table of random numbers. Also, open petri dishes containing nutrient agar were placed at random sampling points along the selected arc. Numerical bacterial dispersion per unit time and the dispersion pattern for the prevalent meteorological conditions were calculated.

Arithmetic-normal dispersion patterns (Figure 4, page 36) were prepared graphically from the colony counts found on the downwind drums and the random number samples after the counts were corrected for individual sampling unit deviations. The colony counts were adjusted to the count that would be obtained if precisely 1 cfm of air was passed onto the drum agar surface (Table 1). The horizontal 50 per cent line on arithmetic-normal paper was assigned both the mean and maximum colony count per sampling period. It was intersected vertically by the downwind count which gave one point on the graph. The random number count was considered a fractional ratio of the maximum count possible, which occured at the 50 per cent line. The fraction was converted to a horizontal percentage by multiplying the ratio by 50 instead of 100. This horizontal percent line was intercepted vertically by the random number colony count, giving the second point on the arithmetic-normal graph. A line drawn through the two points on the graph represented the normalized Gaussian distribution

of airborne bacteria downwind from the plant at the time the samples were taken. The standard deviation was represented at the point where the straight line crossed the 15.8 per cent horizontal value; the mean occurred where the line intercepted the 50 per cent value.

CHAPTER V

OBSERVATIONS AND DISCUSSION

Table 1 lists some of the bacteria which were identified as being common to the aereation tanks of the Northside Sewage Treatment Plant, Oklahoma City, Oklahoma.

TABLE 1

BACTERIA IDENTIFIED FROM THE AEREATION GALLERY OF THE NORTHSIDE SEWAGE TREATMENT PLANT BETWEEN 7-29-70 AND 5-29-71

Aerobacter cloacea	*Klebsiella aerogenes*
Aeromonas liquefaciens	*Lactobacillus helveticus*
Alcaligenes faecalis	*Micrococcus dentrificans*
Bacillus species	*Proteus mirabilis*
Chromobacterium janthenium	*Pseudomonas* species
Citrobacter freundii	*Pseudomonas aeruginosa*
Corynebacterium pseudodiphtheriticum	*Serratia plymuthica*
Corynebacterium xerosis	*Staphylococcus aureus*
Escherichia coli	*Staphylococcus epidermidis*
Paracolobactrum aerogenoides	*Streptococcus faecalis*

Because of the aerobic process utilized in the activated sludge process and the aerobic nature of the samples taken in this study, only aerobic organisms from the aereation gallery of the sewage treatment plant were incorporated into this study. Since this study was confined to those organisms which could directly influence Man, a temperature near the normal body temperature of man was selected for the cultivation of bacteria collected from the plant. These isolates represented organisms which were not normally identified in upwind air samples, therefore their presence in downwind air samples suggested the aereation tanks as their most probable origin.

The three air sampling units (one drum sampler and one vacuum pump) used in this study varied between 6 per cent and 13 per cent of the rated capacity (1 cfm) of the manufacturer's designated rating. The differences in calibrated and designated ratings were corrected for by calibrating the units against a wet-test meter, and therefore did not affect the 1-hour sampling periods of this study. It was noted that this variance could be an important factor in samples of 24-hours or more.

The sampling dates, locations and meteorological parameters taken during each sampling period are presented in Table 2. Four samples were taken on arc A in order to establish some concept of reproducibility and the influence of varied meteorological parameters upon one area of sampling.

TABLE 2

DATES, GRID LOCATION AND METEOROLOGICAL DATA PERTAINING TO AIR SAMPLES TAKEN FROM THE NORTHSIDE SEWAGE TREATMENT PLANT FROM 7-29-70 TO 5-29-71

Date	Wind Direction Site	Random Number Site	Temp. °F.	Rel. Hum. %	Wind Direction	Wind Speed
7-29-70	A-10	--	96	41	SSW	7.0
7-31-70	A- 8	--	85	54	SSE	6.4
8- 4-70	A- 8	--	84	66	SSW	6.9
8- 6-70	A- 8	--	89	49	SSE	11
9-24-70	B- 6	B- 8	70	61	S	11.6
10-22-70	B- 8	B-10	83	53	SSE	17
10-27-70	C-14	C- 3	64	47	S	6.9
3-24-71	C- 2	C- 7	48	47	SE	6.5
3-30-71	D- 9	D- 8	79	29	SW	9.7
4- 8-71	D- 8	D-12	70	30	SSW	16
4-15-71	E- 8	E-17	69	60	SSE	8.6
4-20-71	E- 8	E- 4	77	41	S	8.3
5- 4-71	F- 9	F-12	70	60	SE	14.1
5-14-71A	F-10	F- 5	80	39	S	5.0
5-14-71B	G- 9	G- 3	81	39	S	3.6
5-20-71A	G- 9	G-15	77	36	S	7.6
5-20-71B	H- 8	H-19	78	33	SSE	9.1
5-25-71A	H- 7	H- 3	79	44	SSE	3.8
5-25-71B	I-12	I- 2	84	33	SSW	6.3
5-29-71A	I-12	I-17	66	71	SSE	6.6
5-29-71B	J-10	J-15	70	74	SSE	6.6

In general, there was a drop in colony count with an increase in temperature; however, this drop was not observed unless the temperature exceeded 76° F. A combination of elevated temperature and low relative humidity acted synergistically

to reduce the colony count more than either factor alone.

Table 3 introduces the bacterial distribution as it occurred in upwind and downwind air samples. Bacillus species were found to be the predominating organism both upwind and downwind. These organisms are common soil bacteria, and would be expected to be present in domestic sewage. Alcaligenes faecalis was the second most common organism found downwind. Since the organism was not identified upwind from the aereation tanks, the possibility of using A. faecalis as an ambient air "indicator organism", downwind from the plant studied, is suggested.

Two genera occurred more frequently upwind than downwind. These were Corynebacterium and Aeromonas. Corynebacterium species are widely distributed in nature (31) and Aeromonas species are commonly found in and around water and soil (31). The near proximity of the Oklahoma City Zoo (located in Lincoln Park) may account for the isolation of such organisms as C. bovis, C. equii and Aerobacter cloacae. The occurance of Staphylococcus aureus five times more often downwind than was recorded upwind (Table 3) readily indicates the possibility of the dispersion of primary pathogenic bacteria downwind from an activated sludge sewage treatment plant.

Table 4 indicated that a greater diversity of organisms was present in the ambient air downwind from the aereation tanks than was found in upwind air samples. Numerical

TABLE 3

FREQUENCY DISTRIBUTION OF MICROORGANISMS
IN UPWIND AND DOWNWIND AIR SAMPLES
TAKEN AT THE NORTHSIDE SEWAGE TREATMENT PLANT
FROM 7-29-70 TO 5-29-71

Number of Colonies Observed Upwind	Microorganism	Number of Colonies Observed Downwind
37	Bacillus 'species'	47
0	Alcaligenes faecalis	20
7	Bacillus subtilis	12
0	Escherichia coli	11
0	Achromobacter xerosis	10
2	Staphylococcus aureus	10
3	Aeromonas liquifaciens	8
8	Corynebacterium xerosis	8
0	Klebsiella aerogenes	8
2	Pseudomonas aeruginosa	8
0	Pseudomonas 'species'	7
0	Hafnia group	6
0	Proteus mirabilis	6
0	Proteus vulgaris	6
1	Staphylococcus epidermidis	6
2	Achromobacter iophagus	5
0	Aerobacter aerogenes	5
0	Chromobacterium janthenium	5
1	Citrobacter freundii	5
1	Actinomycetes 'species'	4
0	Corynebacterium bovis	4
1	Flavobacterium 'species'	4
0	Providence, group A	4
0	Aerobacter cloacea	3
1	Corynebacterium pseudodiptheriticum	3
2	Serratia 'species'	3

TABLE 3 Continued

Number of Colonies Observed Upwind	Microorganism	Number of Colonies Observed Downwind
0	Streptococcus faecalis	3
0	Achromobacter 'species'	2
0	Achromobacter mallei	2
0	Alcaligenes marshallii	2
0	Chromobacterium 'species'	2
12	Corynebacterium 'species'	2
1	Corynebacterium equii	2
0	Lactobacillus lactis	2
0	Lactobacillus helveticus	2
2	Micrococcus 'species'	2
0	Micrococcus dentrificans	2
3	Aeromonas 'species'	1
0	Aeromonas formicans	1
0	Alcaligenes 'species'	1
0	Bacillus coagulans	1
0	Citrobacter 'species'	1
0	Klebsiella ozaenae	1
0	Serratia plymuthica	1
0	Neisseria catarrhalis	1
0	Kurtheria 'species'	1
0	Streptococcus 'species'	1

superiority is immediately obvious for downwind samples, as well. It is of interest to the second stage of this study to observe that Serratia marcescens was isolated from air samples a total of six times during the study period which extended from 7-29-70 thru 5-29-71, a total of 10 months. These isolates, having been incubated at 35° C, were colorless upon primary isolation. After having been placed in

TABLE 4

AIRBORNE COLONY COUNTS OBTAINED FROM INDICATED SAMPLING SITES LOCATED AT THE NORTHSIDE SEWAGE TREATMENT PLANT FROM 7-29-70 TO 5-29-71

Date	Colony Count Observed at Wind Direction Sampling Site	Colony Count Observed at Random Number Sampling Site	Colony Count Observed at Control Area Sampling Site
7-29-70	374	--*	27
7-31-70	653	--	26
8- 4-70	655	--	24
8- 6-70	621	--	18
9-24-70	532	487	11
10-22-70	484	403	17
10-27-70	107	22	15
3-24-71	38	30	3
3-30-71	412	383	36
4- 8-71	394	292	218
4-15-71	1,624	38	5
4-20-71	1,472	12	7
5- 4-71	580	118	27
5-14-71A	46	22	29
5-14-71B	86	13	18
5-20-71A	172	144	7
5-20-71B	75	106	12
5-25-71A	98	68	6
5-25-71B	65	15	8
5-29-71A	306	101	14
5-29-71B	53	17	9
$\bar{x}$	421.28	133.59	25.56
S.D.	436.150	152.898	45.042
n	21	17	21

* -- No sample taken.

the genus Serratia, sub-cultures incubated at room temperature produced a pale pink pigment. The production of prodigiosin is quite variable within the genus, and varies within a given species or strain with age, cultural parameters and time (35). Prior to the introduction of the tracer organism, S. marcescens, there were an estimated 1.12×10^{20} bacteria in the aereation gallery. An estimated total of 1.28×10^{19} S. marcescens organisms were introduced into the plant as a tracer organism. Thus, the tracer organism constituted approximately 10 per cent of the bacterial population of the aereation gallery at the beginning of the second stage of the study.

Air samples taken downwind with revolving drum air sampling units, after the 3-hour diffusion period dictated by the sodium flourescene study, indicated an average of 20 colonies of S. marcescens per 60 ft^3 of air were dispersed from the plant. A close figure for bacterial dispersion by the plant into the ambient air per ft^3 would be 3.33 colonies. Open petri dishes were utilized in sampling the ambient air downwind from the aereation tanks after introducing the tracer organism. The variability between colony counts on plates located at the same site for the same length of time gave rise to a dichotomous conclusion, which is perfectly satisfactory as a means of determining whether the tracer organism was or was not dispersed from the aereation gallery into the ambient air.

Statistical analysis of the colony counts presented in Table 4 utilized three one-tailed paired 't' tests. These were: (a) wind direction colony counts compared to control area colony counts, (b) random number colony counts compared to control area colony counts and (c) the wind direction colony counts compared to random number colony counts. Tests 'a' and 'b' indicate that a significant difference existed between the colony counts compared at $P < 0.025$. Test 'c' showed a significant difference between the colony counts existed at $P < 0.050$. Therefore, statistical analysis confirms that there was a greater concentration of bacteria downwind than was found upwind in this study.

The use of arithmetic-normal graphs to represent an estimation of the dispersion pattern from limited observation are represented in Figure 4. By utilizing these graphs in the analysis of field data, it is felt that a more meaningful analysis of the distribution patterns of the study may be accomplished as the mean, maximum and standard deviation are readily obtained from a straight line through the reference points on the graph. The limitations of this introductory presentation are readily apparent, since only two reference points are available. However, with four or more sample points and the utilization of "least square" calculated values, a comprehensive concept of dispersion from a point of origin seems readily available.

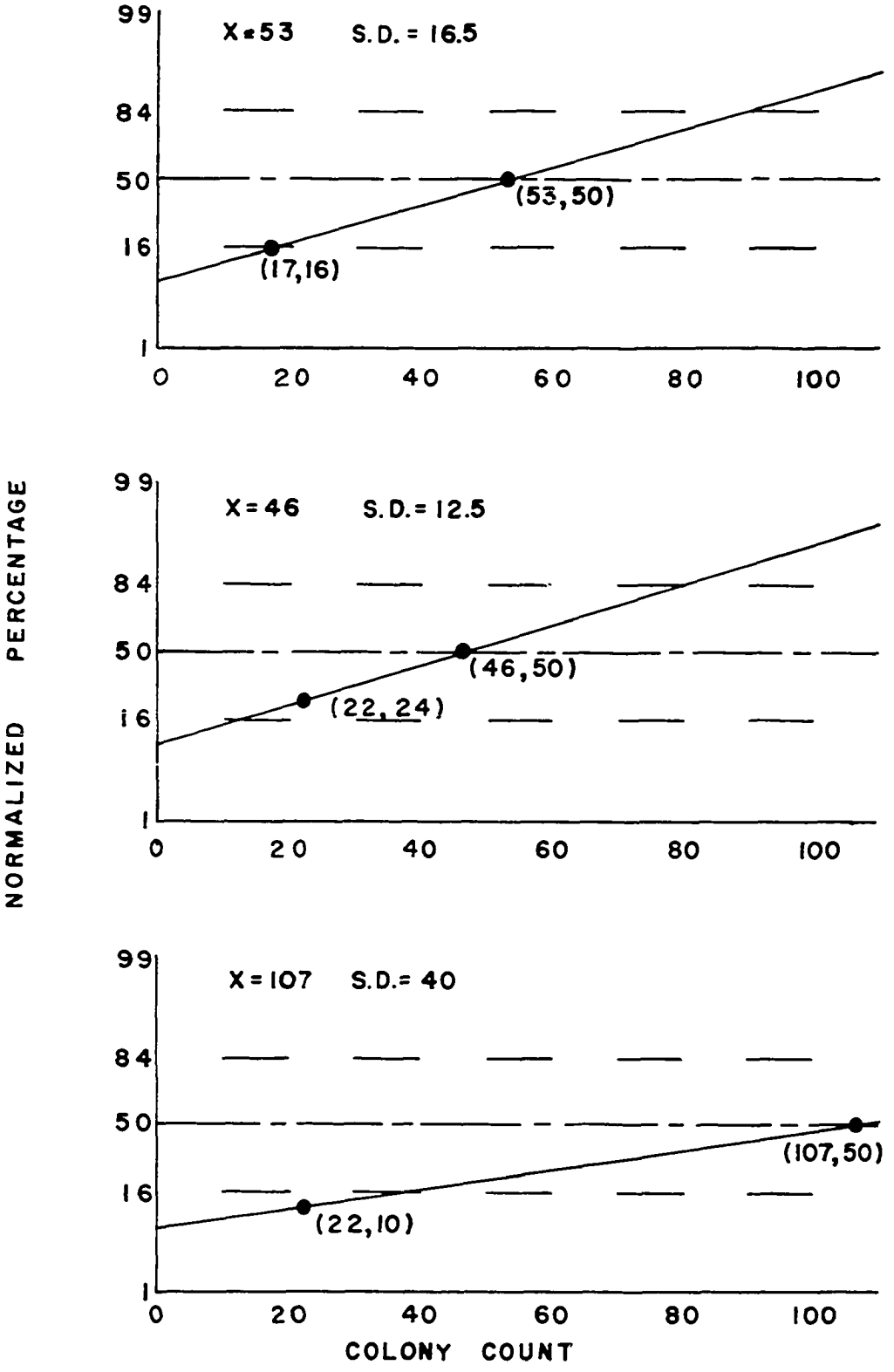

Figure 4. Some representative arithmetic-normal distribution graphs.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This research was designed to investigate the dispersion of bacterial organisms into the ambient air in the vicinity of an activated sludge plant. Two approaches to the problem were employed: (a) the identification of bacteria within the aereation tanks of the plant and the isolation of these bacteria downwind from the tanks where they did not occur upwind, and (b) the introduction of a tracer organism into the tanks in order to establish that these organisms were being dispersed into the downwind ambient air from the tanks. A simple but axcessible means of plotting dispersion patterns from limited data has been introduced.

Based on the results of this study, the following conclusions have been drawn.

1. Bacteria which are common to an activated sludge plant's aereation gallery can be found in the ambient air downwind from that gallery. At a distance of 100 ft downwind from the aereation gallery, as many as 1,472 colonies per 60 ft^3 of

air were obtained, while the upwind ambient air control sample produced only 14 colonies for the same sampling period. This represents a difference of over 100 times the concentration found in normal ambient air for the same period of time. At a distance of 160 ft downwind, the bacterial count was still nearly 22 times as great as that obtained upwind.

Although no proven primary pathogen was isolated in this study, the isolation of many airborne bacteria downwind from the plant, having similar properties to primary pathogens, suggests that an occupational hazard may exist for plant personnel and residents in close proximity to the plant. Microorganisms other than bacteria (virus, fungi, Protozoa and Helminth eggs) may also be dispersed into the air downwind from the plant.

2. The above findings were confirmed when a tracer organism having similar properties to those of the bacteria common to the activated sludge flora was introduced in concentrations of approximately 10 per cent of the total number of bacteria within the aereation gallery. Before the introduction of the tracer culture, only six isolates of Serratia were noted in a period which covered 10 months of sampling both upwind and downwind: however, as

many as 140 of the tracer organisms were collected downwind in a 1-hour period after the introduction of the cultures into the aereation gallery. Bacteria not specifically adapted to survival in ambient air remained viable in air samples taken downwind from the aereation tanks.

3. The isolation of C. bovis, C. equii and Aerobacter cloacae downwind from the aereation tanks suggests that these organisms may constitute a third series of tracer organisms which had passed through, or over, the sewage treatment plant, having been introduced from the Oklahoma City Zoo (located approximately three-blocks from the plant).
4. A readily available estimate of the mean and standard deviation of the Gaussian distribution for a given set of conditions can be determined from arithmetic-normal graphs.

It is recommended that a comparative study of the antibody components of the serum of persons who are exposed to abnormal bacterial concentrations, in conjunction with epidemiological studies as to the resistance or susceptibility to common infections by such persons, will give an insight into the hazards of downwind exposure as well as the mechanism of acquired immunity. Methods of retarding dispersion of microorganisms from aereation tanks would be of value. Studies to define and monitor the normal (and abnormal)

micro-flora of ambient air (including allergins) present challenging areas of research.

A comparative study between presently accepted methods of establishing dispersion patterns, which require highly trained technical personnel, with those which might be readily available to a greater number of health workers by utilizing adaptations of arithmetic-normal graphs (when the limitations of arithmetic-normal graphs are established) is definitely recommended.

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APPENDIX

METHOD OF STATISTICAL ANALYSIS OF DATA

TABLE 5

AN EXAMPLE OF THE STATISTICAL TREATMENT USED FOR COMPARING VARIOUS UPWIND AND DOWNWIND COLONY COUNTS

Wind Direction Colony Count	Control Area Colony Count	Wind Direction Count minus Control Area Count
374	27	347
653	26	627
655	24	631
621	18	603
532	11	521
484	17	467
107	15	92
38	3	35
412	36	376
394	218	176
1,624	5	1,619
1,472	7	1,465
580	27	553
56	29	17
86	18	68
172	7	165
75	12	63
98	6	92
65	8	57
306	14	292
53	9	44
SX= 8,847	SX= 537	SX= 8,310
n= 21	n= 21	n= 21
$\bar{x}$= 421.28	$\bar{x}$= 25.56	$\bar{x}$= 395.72
		SX^2= 7,155,094

Symbols used in statistical analysis of data.

SX ___ sum of figures

n ___ number of figures in column

$\bar{x}$ ___ mean

SX^2 ___ sum of figures squared

$\&_2$ ___ variance

S E ___ standard error

$\bar{x}_1$ ___ mean of column one

t ___ 't' value for test

$\bar{x}_2$ ___ mean of column two

P ___ probability of error

By using the following series of equations, the 't' value of the three statistical analysis performed were derived.

$$\&_2 = \frac{SX^2 - \frac{(SX)^2}{n}}{n-1} \longrightarrow S\,E = \sqrt{\frac{\&_2}{n}} \longrightarrow t = \frac{\bar{x}_1 - \bar{x}_2}{S\,E}$$

When the wind direction colony counts were compared to the control area colony counts, the 't' value was 4.124. With 20 degrees of freedom, a table of 't' distributions gives a value of 1.725 at the 5% level; *P<0.95 (**P<0.995).

In comparing the random number colony counts with the control area colony counts, the 't' value was 3.706. With 16 degrees of freedom, the 't' value given at the 5% level is 1.746. (*P<0.995).

A comparison of the wind direction colony counts with the random number colony counts gave a 't' value of 2.089 at the 5% level with 16 degrees of freedom. *P<0.95 (**P>0.975).

In all cases, the null hypothesis was rejected if the 't' value was equal to or greater than the 't' value given for 95%.