Health Significance of Airborne Microorganisms from Wastewater Treatment Processes Part I: Summary of Investigations

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Source: *Journal (Water Pollution Control Federation)*, Dec., 1975, Vol. 47, No. 12 (Dec., 1975), pp. 2741-2757

Published by: Wiley

Stable URL: http://www.jstor.com/stable/25038442

Health significance of airborne microorganisms from wastewater treatment processes

Part I: Summary of investigations

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The measurement of the emission and airborne spread of viable microorganisms from wastewater collection, treatment, and disposal processes has been the subject of several field studies. The underlying purpose of these investigations was to evaluate to what extent viable aerosols may represent a health risk to wastewater treatment plant workers and those residing in the vicinity of the plants or otherwise exposed to the aerosols.

The following basic premises are common to most of the investigations:

1. Human pathogenic microorganisms are known to be present in wastewater in large numbers at any stage of handling.
2. Most modern wastewater treatment processes mechanically assist in the aerosolization of the liquid wastewater by air injection, spraying, or splashing.
3. The resultant aerosols very likely contain viable microorganisms, including pathogens.
4. Airborne microorganisms are known to survive and to be carried considerable distances by wind currents.
5. Urban development has placed large populations in closer proximity to treatment plants in recent years.
6. Airborne pathogens from wastewater may therefore represent a health hazard to plant personnel and nearby residents.
7. Therefore, measurement of the airborne emission and dispersion of viable microorganisms by concentration or type of organism or both may provide an indication of an associated health hazard.

These investigations have demonstrated that many types of bacteria are emitted to the air from wastewater during almost any stage of collection, treatment, or disposal and that they remain viable and airborne for considerable distances downwind. However, interpretation of the results of these studies in terms of health hazards has been inconclusive. Those investigators who did make conclusions regarding health hazards on the basis of their data did so generally by inference. For example, the recovery of index organisms such as coliforms from the air some distance from the source and in a respirable particle size range was frequently taken to be indicative of a health hazard from inhalation of respiratory pathogens. Conversely, the failure to recover abnormally high concentrations of airborne microorganisms downwind from a wastewater plant has been interpreted as the absence of a significant health risk from this source.

Because of the current interest in the possible health effects from viable aerosols from wastewater, particularly from landspraying operations, it seems appropriate to summarize and evaluate the results of investigations to date. This review has been prepared in two parts. Part I summarizes in detail the results of field investigations and related literature on the subject. Part II evaluates these findings in terms of health significance to wastewater plant workers and others exposed to wastewater aerosols and directions for future investigations.

Sixteen published reports representing

13 separate field studies, all that could be found in English, form the primary focus of this review.[1-16] Six of these reports are master's or doctoral theses or articles based on them. Other field studies available only in abstract or summary form [17-25] have been cited briefly and have been included to supply a more complete bibliography. Laboratory studies of the generation of bacterial aerosols from wastewater,[26, 27] field studies of protein-bearing particles generated from wastewater,[28-31] and several articles that discuss or attempt to assess the health risks associated with airborne microorganisms from wastewater on the basis of health surveys or models [32-41] have also been reviewed. Other literature has been cited where appropriate.[42-54] (Additional references will appear in Part II.)

For convenience, some terms related to aerosol characteristics are defined as follows: A viable particle is an airborne particle containing living matter as demonstrated by growth or replication under defined conditions. In this review, a viable particle is one containing at least one bacterial cell capable of forming a colony under conditions selected by the investigator. The viable aerosol is the mass or cloud of viable particles emanating from a source. The diameter of a viable particle refers to its aerodynamic (equivalent) particle diameter, which is the diameter of a unit-density sphere, which behaves in the same manner as the particle with respect to an aerodynamic characteristic, usually, settling velocity. The aerodynamic particle diameter is often used because it more accurately reflects the behavior of an airborne particle and is usually more easily measured than is its physical diameter. The diameter of an aerosol as a whole refers to the geometric mean of the diameters of its individual particles.

SUMMARY OF LITERATURE

Direct field investigations. In 1907, Horrocks [1] demonstrated the aerosolization and airborne spread of bacteria from wastewater flowing in sewers. He seeded the wastewater with *Bacillus prodigiosus* and recovered this organism on agar settling plates placed in branch ventilation risers as high as 50 ft (15 m) above the flowing wastewater, although the branch risers were upstream from the flow of seeded wastewater. The organism was neither recovered on control plates nor was it recovered from branches separated from the seeded wastewater by running traps. Horrocks also recovered coliforms and *Bacillus typhosus* on agar settling plates in branch risers. He concluded that bacteria could be injected into the air by bursting bubbles at the surface of the wastewater, by reentrainment of dried particles from sewer walls, and, probably, by the ejection of droplets from the flowing wastewater. He also concluded that air inlets to a sewer system may be a source of danger when they are placed near the ground level.

Following Horrocks' study, there were no further published reports on airborne contamination from wastewater works until Fair and Wells [2] revived the subject in 1934. They observed that the idea that wastewater may contaminate the air had been abandoned many years earlier on good authority, but they believed that the subject should be reexamined in view of then recent developments both in wastewater treatment processes and in concepts of airborne infection.

The "good authority" may have been the 1917 final report of the American Public Health Association Committee on Standard Methods for the Examination of Air.[42] This report stated that the number of bacteria in the air does not seem to be a factor of any great sanitary significance but may be of interest in special cases, as in the study of dairy conditions. Another reason for the lack of interest in airborne contamination may have been Winslow's conclusion [17] that bacteria liberated into the air by splashing wastewater are present in such small numbers that it would be possible to breathe sewer gas from a pipe for 24 hr without becoming infected. There is no record that this hypothesis was ever tested.

In any event, Fair and Wells[2] assayed total and coliform bacteria in air at and at an unspecified distance from activated sludge aeration tanks and trickling filters by means of the Wells air centrifuge.[43] They reported total bacteria recoveries of up to 22.3 organisms/cu ft (787 cu m) at the sources and up to 3.9 organisms/cu ft (138/cu m) away from the sources. Coliform recoveries, as determined by eosin methylene blue (EMB) agar counts, were reported to be up to 2 organisms/cu ft (71/cu m) at the sources and less than 1 organism/cu ft (35 organisms/cu m) away from the sources.

Interestingly, the highest count of 22.3 organisms/cu ft (787/cu m) of air was obtained by sampling air confined in a glass dome suspended over the surface of the activated sludge aeration tank. This exact procedure is described in no other report, although Kenline[10] outlines reasons for not sampling in this manner and Glaser and Ledbetter[31] used a similar procedure in their assay of protein-bearing aerosols from wastewater for which viability was not determined. Fair and Wells[2] made no conclusions regarding sanitary hazards of airborne bacteria from wastewater, stating that the extreme dilution of the contaminated air must be considered.

In 1957, Merz[3] investigated the hazards associated with sprinkling treated wastewater onto a golf course. Air was sampled downwind from a covered sedimentation basin, a wastewater aeration tank, and a sprinkler by using a sampling instrument with a rectangular orifice that impinged air onto the surface of liquid collection media. The sampler fluid was assayed for coliform organisms. Coliforms were reported to have been recovered only downwind from the sprinkler and close enough to it [135 ft (41.1 m)] that the spray could be felt. Merz concluded that hazards from sprinkling wastewater were limited to direct contact with unevaporated droplets. Merz's study (now out of print) is the only published U. S. field study that could be found that addressed airborne microorganisms from land application of wastewater although some foreign language articles and unpublished material do address the subject. Schultze[18] recovered *Escherichia coli* on settling plates located downwind from sprinklers discharging raw wastewater onto crops. Reploh and Handloser,[19] by using agar settling plates, found airborne dispersion of coliform bacteria downwind from sprinklers discharging raw wastewater that was not disinfected. They estimated that the viable aerosol could be carried 400 m downwind by a 5-m/sec wind and recommended that large land areas and the planting of hedges be used as safety measures.

Bringmann and Trolldenier,[20] by using Endo agar settling plates, investigated the airborne spread of bacteria downwind from sprays discharging settled wastewater that was not disinfected. They found that the downwind travel distance of the viable aerosol increased as relative humidity and wind speed increased and decreased as ultraviolet radiation increased. They estimated that coliform organisms may remain viable as far as 400 m downwind from the source under conditions of darkness, 100 percent relative humidity, and a wind speed of 7 m/sec. Sepp[21] measured the airborne spread of total and coliform bacteria downwind from sprayers dicharging ponded and chlorinated activated sludge tank effluent. Coliform bacteria were recovered as far as 10 ft (3.0 m) downwind from the spray limits in a dense brushy area and up to 200 ft (61 m) downwind from the spray limits in a sparsely vegetated area. Shtarkas and Krasil'shchikov[25] recovered bacteria on settling plates 650 m downwind from sprinklers discharging settled wastewater and recommended a 1,000-m sanitary zone around such installations.

In addition, Sorber *et al.*[35, 37] and Sorber[36] have summarized the literature on airborne microorganisms (including viruses) from the land application of wastewater and have postulated the health implications of this practice through a mathematical model (to be discussed later).

Albrecht[4] studied the airborne spread

of bacteria downwind from a trickling filter plant by using the Andersen six-stage impactor sampler,[44] the Wells air centrifuge, and the midget impinger.[45] Samplers were placed on the ground during sampling. Initially, Albrecht also sampled downwind from the plant influent line, sedimentation tanks, sludge digester, and sludge drying beds. He concluded that the primary sources of the bacterial aerosol were the two trickling filters, the other units being minor contributors by comparison. He then assayed airborne *E. coli*, *Aerobacter aerogenes*, and total bacteria on a grid pattern (with an upwind control) downwind from the filters to a distance of 300 ft (91 m).

Albrecht reported recovering coliform organisms as far as 100 ft (30.5 m) downwind from the filters. The maximum coliform recoveries farther than 3 ft (0.9 m) from the edges of the filters were 6 *E. coli* and 5 *A. aerogenes*/cu ft (212 *E. coli* and 177 *A. aerogenes*/cu m). The number of total bacteria recovered was reported to be as high as 144/cu ft (5,080/cu m) at one filter's edge and up to 80/cu ft (2,820/cu m) at a downwind distance of 50 ft (15.2 m). In 4 of 18 samples, the upwind control counts were equal to or higher than recoveries immediately downwind from the filters, but no *E. coli* and few *A. aerogenes* were recovered upwind. Albrecht discussed no additional possible sources for the recovered organisms other than the filters.

Albrecht recorded wind speed and direction, temperature, and relative humidity. He was unable to demonstrate any correlation between the distance of coliform spread in air and the air temperature or relative humidity. He cited the work of Imhoff and Fair[46] and Halvorson *et al.*,[47] which shows that an updraft occurs in a trickling filter when the air temperature is at or below the wastewater temperature. Albrecht, postulating that updraft conditions would enhance the spread of airborne bacteria from the filter, plotted downwind *E. coli* recovery under filter updraft conditions as a function of wind speed and observed that a positive relationship existed. He concluded that conditions of filter ventilation are related to the existence and range of spread of airborne bacteria emitted from the filter and that wind plays an important role in the distance of spread.

Four of the 14 sample sets were taken in darkness. Albrecht does not discuss this point, but no differences in results are apparent. No particle size distributions of Andersen sampler recoveries are presented. Other than the observation that filter updraft conditions increase the downwind spread of coliforms, there is no discussion by Albrecht of horizontal or vertical dispersion characteristics of the viable aerosol.

In preliminary laboratory tests, the viable particle recovery levels of the Wells air centrifuge and the Andersen six-stage impactor were closely comparable, while those of the midget impinger were 31 times higher. In the only two field samples in which all three samplers were used together, the same pattern held. Higgins[26] regards these findings as being characteristic of an aerosol with a large number of organisms per particle. The impingers were placed 21 ft (6.4 m) from the edge of the filter during sampling and could have been collecting unevaporated spray.

Ledbetter and Randall[5] and Ledbetter[6] studied downwind dispersion of bacterial aerosols from activated sludge aeration tanks by using as samplers agar settling plates held 4 ft (1.2 m) above ground for 3 min with the agar surface facing into the wind at a 30-deg angle. Samples were taken at 20, 50, and 100 ft (6.1, 15.2, and 30.5 m) downwind and at 20 ft (6.1 m) upwind. Both nutrient agar and EMB agar plates were exposed, and total coliform colony counts were differentiated.

Ledbetter and Randall[5] found that the number of colonies recovered decreased rapidly with the distance from the source. Counts on nutrient agar were 32 colonies at 20 ft (6.1 m) downwind from the tanks and six colonies at 100 ft (30.5 m) downwind from the tanks. Counts on EMB agar similarly decreased from 14 to 2, and coliform colonies decreased from 5 to 1 in the same distance. All of the above counts are net counts after subtraction of simultaneous upwind control counts, which were not reported. By using the regression analysis method, Ledbetter and Randall calculated the tank aerosol emission levels to be 745 colonies above controls for nutrient agar and 227 for coliform organisms at an apparent plate exposure time of 3 min. They also calculated the half-life for total airborne bacteria to be 0.38 sec 20 ft (6.1 m) downwind from the tanks and attributed the short half-life to die-off and dispersion. The half-life of coliforms was found to be shorter than that of total bacteria.

No correlation was found between the number of colonies recovered and the relative humidity or air temperature. Ledbetter and Randall concluded that passage over the aeration tank caused an increase in the bacterial population of the air, which persisted for a considerable distance, the distance being heavily dependent on wind speed. Wind speeds were obtained from an airport 3.5 miles (5.6 km) away. There was no discussion of whether the higher recoveries at higher wind speeds may have been a result of shorter transit time in air, greater impact velocity onto the agar surfaces, or some other phenomenon. However, Randall and Ledbetter [9] reexamined the data later and found a dual bacterial die-off rate characterized by a very short and very rapid initial die-off rate, followed by stabilization and a very slow die-off rate for the more resistant organisms. By suppressing the wind speed factor, they also found a trend toward longer bacteria survival at higher relative humidities.

Napolitano and Rowe [7] investigated the emission and downwind spread of coliform organisms from various units in four activated sludge and high-rate trickling filter plants by using the Andersen six-stage impactor. Total counts recovered on EMB agar were reported as coliform organisms. Initially, 5-min samples were collected at the primary sedimentation tanks, preaeration tanks, aeration tanks, and final sedimentation tanks of the activated sludge plants and at the primary and final sedimentation tanks and filters of the trickling filter plants. The trickling filters were reported to yield twice as many coliforms as the other trickling filter plant units, and the activated sludge aeration tanks were the major contributors of aerosols at the activated sludge plants.

After establishing to their satisfaction that the activated sludge aeration tanks and the trickling filters were the primary sources of viable airborne coliforms at the respective plants, Napolitano and Rowe took samples downwind from these units at distances of 0, 50, 100, and 150 ft (15.2, 30.5, and 45.7 m). Five-min samples, [each comprising 5 cu ft (0.14 cu m) of air] collected at the activated sludge aeration tanks gave recoveries of 14 and 109 coliform colonies; recoveries 150 ft (45.7 m) downwind were 1 and 154 colonies. In two similar samples taken immediately downwind from the trickling filters, 15 and 149 colonies were recovered; at 150 ft (45.7 m) downwind, two other samples of 1 and 20 colonies were recovered.

No particle size data were presented, but Napolitano and Rowe stated that 50 percent of the particles emitted were less than 5 μ in diam and could be considered a health hazard. They also pointed out that there were differences among the four plants in the quantity and quality of wastewater and in the weather conditions during sampling; they suggested caution in accepting the results of research on microbial air pollution from wastewater treatment plants.

Ladd [8] examined the generation of aerosols from a preaeration tank, a trickling filter, and an activated sludge tank by using the Andersen drum sampler [48] and the Andersen six-stage impactor to assay viable bacteria and a sequential membrane filter sampler to assay protein-bearing aerosols. Samples were collected immediately downwind from the various units, and control samples were taken upwind. Viable particle recoveries per cubic foot downwind were up to 351 (12,400/cu m) at the preaeration unit, 146 (5,160/cu m) at the activated sludge unit, and 77 (2,720/cu m) at the trickling filter after subtracting colony recoveries on upwind controls, which varied from 28 to 86 colonies/cu ft (1,000 to 3,040/cu m) of air sampled.

Ladd examined the relationships between the rate of viable particle emission as measured by colony recovery and air temperature, relative humidity, wind speed, and wastewater flow rate. Linear regression analyses indicated that viable particle emission rates increased slightly with increased wastewater flow rates. Emission rates from the preaeration tank and the trickling filter decreased as air temperature increased but increased with air temperature at the activated sludge tank. As wind speed increased, viable aerosol recoveries at the preaeration tank decreased markedly but recoveries at the other two units increased slightly. As relative humidity increased from 15 to 45 percent, there were sharp declines in recoveries at the trickling filter and the preaeration tank. However, recoveries at the activated sludge tank, at which all sampling was done at higher relative humidities (45 to 85 percent), decreased only slightly as relative humidity increased. Ladd concluded that his data showed that bacteria emission rates were affected by the four variables mentioned above; at the same time, he acknowledged that the wide dispersion of results indicated other influencing factors. Wind speed and relative humidity measurements were taken from nearby airport records.

As an indication of the potential hazard of viable wastewater aerosols to man, Ladd examined 180 collected colonies on differential media and found 53 coliform colonies and 64 colonies that produced beta-hemolytic reactions. Both types were recovered downwind from all units. No differential examinations were performed on colonies recovered upwind.

Ladd released cultures of *Bacillus subtilis* var. *globigii* into sewers as far as 5 miles (8 km) from one plant and recovered the organisms from the air downwind from the preaeration tank 5 hr after the organisms were released; the peak recovery level was 15 colonies/cu ft (530/cu m). Not one of the species was recovered from upwind controls, and not one was recovered more than 10 hr after being released. Protein-bearing material was recovered from the air on membrane filters placed downwind from the activated sludge and preaeration tanks. No quantities were reported, and no protein samples were taken upwind. Ladd considered that the Andersen drum sampler gave a more representative sample of the viable particle emissions than did the Andersen six-stage impactor because the former sampled continuously for long periods. No particle size data from the Andersen impactor were reported. Ladd concluded that harmful bacterial aerosols are emitted from wastewater treatment plants and that these emissions could be harmful to plant operators and those living nearby.

Randall and Ledbetter [9] sampled for airborne bacteria downwind from two activated sludge aeration tanks and one grit chamber at two treatment plants, one of which practiced prechlorination. The Andersen six-stage impactor, the all-glass Millipore impinger, and agar settling plates were used. Procedures were the same as those outlined in a previous work.[5] Samples were taken immediately downwind from the units at 2 ft (0.6 m) above the liquid surface. The Andersen impactor recovered 870 colonies/cu ft (30,700/cu m), the impinger recovered 1,170/cu ft (41,300/cu m), and the agar plates recovered 220 colonies in an unspecified time period (probably 3 min on the basis of previous work). Upwind colony recoveries on the Andersen impactor averaged 6 to 8/cu ft (210 to 280/cu m) of air sampled.

Randall and Ledbetter found that the settling plates recovered as many different groups of Enterobacteriaceae as did the Andersen impactor, but not as many different types of other enteric organisms. In samples taken adjacent to the aeration tanks, large numbers of indole-positive *Klebsiella* were recovered. Because these are known respiratory tract pathogens, Randall and Ledbetter calculated that a person standing 5 ft (1.5 m) downwind from an aeration tank would be expected to inhale a viable *Klebsiella* every two breaths if it is assumed that each viable particle contained one viable *Klebsiella* organism. Randall and Ledbetter reported that 10.5 percent of the total bacteria recovered were *Klebsiella, Aerobacter,* or *Proteus* organisms, all of which are potential pathogens of the respiratory tract. Members of the Providence group were also recovered. Despite repeated long-term sampling, neither *Shigella* nor *Salmonella* sp was recovered from air, a finding that Randall and Ledbetter expected in view of the relatively small numbers of these genera in wastewater.

Randall and Ledbetter also found that capsulated organisms (*Klebsiella, Aerobacter*) were more suited to survival in air than acapsulate organisms (*Escherichia*), based on observation of a less rapid die-off rate in the former group as downwind distance from the sources increased. In an attempt to evaluate the loss of virulence in airborne *Klebsiella,* organisms recovered at various distances downwind were examined for a loss of capsulation capability. It was found that capsule production capability in organisms recovered 50 to 100 ft (15.2 to 30.5 m) downwind from the aeration tank was 57 to 63 percent of that of organisms recovered immediately downwind.

Randall and Ledbetter found little difference in the recoveries of airborne bacteria near the aeration tanks of plants that did or did not practice prechlorination. They did observe larger fractions of *Klebsiella* in air samples taken near a grit chamber (raw wastewater) than in air samples taken at the aeration tank. They noted the probable influence of other sources in the plant on airborne bacteria recovery, observing that Enterobacteriaceae were recovered from control samples taken upwind from the aeration tank but not from air samples taken upwind from the entire plant.

Randall and Ledbetter calculated that 40 percent of the viable airborne bacteria near an activated sludge aeration tank are associated with an aerosol particle size permitting lung penetration (5 μ in diam or less) and that this fraction increases to 70 percent at a distance of 20 ft (6.1 m) downwind. Randall and Ledbetter concluded that there exists a definite possibility of airborne infection from activated sludge units and that *Klebsiella* are the best indicators of bacterial air pollution from wastewater sources.

Kenline [10] and Kenline and Scarpino [11] investigated the emission and spread of airborne bacteria from activated sludge aeration tanks and from two small extended aeration plants. The Andersen six-stage impactor and agar settling plates were used to assay total viable bacteria and Enterobacteriaceae at ground level for distances up to 150 ft (45.7 m) from the activated sludge units and 61 and 75 ft (18.6 and 22.9 m) from the extended aeration plants.

These investigators used stationary sampling points and did not move them to maintain sampling locations directly downwind. Instead, Kenline and Scarpino used a dispersion model (after Turner [49]) and their observed airborne bacterial concentrations to calculate the emission rate of airborne bacteria from the tanks as well as the dispersion and fate of the bacteria. They calculated the mean emission rate of viable bacteria to be 440 organisms/sec/sq m of tank surface, presuming 1 viable organism/particle. Kenline [10] believed that this presumption was supported by the literature and by observations on the particle size distribution of viable organisms as determined by Andersen impactor counts. Kenline listed the difficulties in obtaining a representative sample as his reason for not measuring the bacterial emission rate directly from the aeration tank surface.

Kenline and Scarpino found a rapid depletion rate of viable organisms as the aerosol moved downwind from the tanks and calculated that the primary depletion mechanisms were die-off (half-life = 13.8 sec) and diffusion, which exerted a greater effect with increasing downwind distances.

Deposition of viable particles was found to have a lesser and nearly constant effect on aerosol depletion at distances greater than 35 ft (10.7 m) from the edges of the tanks. By using bacteria counts on agar settling plates placed beside the Andersen impactor during sampling, Kenline and Scarpino calculated the viable particle deposition rate to be 1 cm/sec at distances greater than 15 ft (4.6 m) downwind. Within 15 ft (4.6 m) of the tanks, the deposition rate was much greater and was attributed to spray deposition.

Kenline,[10] observing that the emitted droplets evaporated in less than 1 sec, considered that evaporative stress [17] was primarily responsible for the die-off of viable airborne bacteria and that the effects of radiation were too slow to account for the observed rapid die-off. Kenline was unable to find any effect of relative humidity on the die-off rate, and he attributed this to the complex relationship between the two, as has been reported extensively in the literature. Kenline's data suggested that increased air temperature resulted in a lower die-off rate by a factor of eight as the air temperature increased from 37°F (3°C) to 82°F (28°C). The die-off rate did not appear to change as the distance from the tanks increased from 50 to 150 ft (15.2 to 45.7 m), evidently stabilizing after a few seconds.

Kenline found a mean upwind bacterial concentration of 3 organisms/cu ft (106/cu m) of air, a level equal to the concentrations both observed and calculated at a downwind distance of 150 ft (45.7 m). On this basis, it was concluded that the effects of the aeration tanks did not normally extend more than 150 ft (45.7 m). It was also concluded that the initial concentrations of bacteria are reduced by 90 percent within 100 ft (30.5 m) downwind of the aeration tanks under average weather conditions. Under conditions more conducive to the generation and spread of viable aerosols, the calculations show that airborne bacterial concentrations may reach 10 organisms/cu ft (350/cu m) at 200 ft (61 m) from the tanks. The maximum counts observed were 60 organisms/cu ft (2,100/cu m) at 50 ft (15.2 m), 5.7 (200/cu m) at 100 ft (30.5 m), and 1.5 (53/cu m) at 150 ft (45.7 m) from the aeration tank gallery. However, because samples were not always taken directly downwind, the calculations for downwind concentrations are more valid for comparative purposes than are the observed counts.

The data did not indicate that any appreciable plume-rise effect exists at the aeration tanks, the ground level bacterial concentrations being consistently higher than those at 5 and 8 ft (1.5 and 2.4 m) above the ground.

The emission and spread of bacterial aerosols from the activated sludge tanks and two small extended aeration plants were compared by Kenline and Scarpino.[11] At the extended aeration plants, the calculated viable aerosol emission rate was lower (120 bacteria/sec/sq m of tank surface), the half-life was longer (32 sec), and the deposition rate was higher (3.8 cm/sec). Airborne organisms were recovered up to 75 ft (22.9 m) from these plants. Kenline [10] calculated that the effect of the plants on airborne bacterial concentrations would not extend past 100 ft (30.5 m) and concluded that 100 ft (30.5 m) may be an adequate "sanitary zone" for such plants.

Escherichia, *Klebsiella*, and *Aerobacter* genera were recovered from the air near the activated sludge aeration tanks. The Enterobacteriaceae survival rate was found to be only 13 percent of that of the total bacterial aerosol. It was concluded that a total airborne bacterial count would be a faster and more reliable indicator of bacterial air pollution from an activated sludge unit than a specific index organism. The Andersen six-stage impactor was found to be a more useful sampler for this type of assay than the Andersen drum sampler or the midget impinger.

Kenline [10] concluded that the dispersion equation he used may be confidently applied to predict airborne bacterial concentrations under conditions not directly investigated in his study (including nighttime conditions) and that the parameters determined (emission rate, deposition rate, and half-life) may be applied as a first approximation of such conditions. He observed that the dispersion model resulted in the calculation of a theoretical upper limit on the bacterial emission rate from the tanks, regardless of the size of the aeration gallery, because the die-off and the deposition rates over the gallery would eventually equal the emission rate. In addition, Kenline reported recovering four to seven organisms/cu ft (140 to 250/cu m) of air when the wind was blowing toward the samplers from a raw wastewater inlet and concluded, as did Horrocks,[1] that flowing raw wastewater can emit a viable bacterial aerosol.

Kenline[10] made no direct conclusions from his data regarding health hazards. He calculated that the average recovery 50 ft (15.2 m) downwind from the activated sludge aeration tanks was 300 bacteria/cu m in the size range permitting lung penetration, which would result in the inhalation of 2 bacteria/min if a normal breathing rate were assumed. He concluded that this seemed to be of little practical significance.

Adams and Spendlove,[12] by using the Andersen six-stage impactor, measured the nighttime spread of airborne bacteria from 50 ft (15.2 m) to 0.8 mile (1.3 km) downwind from a large and a small trickling filter plant. Control samples were taken upwind. Viable particles per cubic meter recovered on casitone agar were reported as total bacteria, and those recovered on Endo's medium and EMB agar were reported as coliforms. The Adams and Spendlove paper is unique in that it reports recovery of airborne coliform organisms, attributed to the trickling filter, as far as 0.8 mile (1.3 km) downwind from the larger of the two plants. At this distance, 3 and 4 coliforms/cu m were recovered on two samples, with an upwind recovery of 1 coliform/cu m. Coliform recoveries up to 50 yd (45.7 m) from the filters ranged from 105 to 934 viable particles/cu m; at 300 yd (275 m), they ranged from 70 to 193 viable particles/cu m; and at 600 yd (550 m), they ranged from seven to 109 viable particles/cu m.

Adams and Spendlove considered that the concentration of airborne coliform organisms near the filter was most affected by the size of the source and the wind speed and that high wind speeds, high relative humidity, darkness, and low air temperatures would be expected to permit the greatest viable particle recoveries both near the plant and at greater distances downwind. They did not attempt a detailed examination of these variables. However, the limited data indicate a trend toward higher coliform recoveries at higher relative humidities. The particle size distributions of the aerosols were not presented, but the highest colony counts were reported to be obtained on the second, third, and fourth stages of the Andersen impactor. This indicates that the aerosols are in a size range permitting both bronchia deposition and upper respiratory tract retention with a possible hazard from subsequent swallowing.

Adams and Spendlove concluded that aerosolized coliform organisms from wastewater treatment plants may indicate a public health concern; in addition, they stated that their study was qualitative and that further identification of other bacterial, viral, and fungal aerosols generated by wastewater treatment facilities should be attempted. The data presented on total bacterial counts are not discussed in detail. In some cases, upwind control counts were higher than downwind counts; this fact supports the possibility of interference from other bacterial aerosol sources.

King[13] and King *et al.*[14] examined the spread of airborne bacteria downwind from activated sludge aeration tanks by using the Andersen drum sampler. They recovered a mean of 9 viable particles/cu ft (320/cu m) from air samples taken at ground level, a distance of 20 ft (6.1 m) directly downwind from the tanks, and a mean of 7 viable particles/cu ft (247/cu m) from air samples taken at intervals from 20 to 200 ft (6.1 to 61 m) downwind. Recoveries on upwind control samples averaged 0.4 viable particles/cu ft (14/cu m). In the single sample taken 200 ft (61 m) downwind from the gallery, 53 colonies/60 cu ft (31/cu m) were recovered. Recoveries from samples taken up to 10 deg horizontally on either side of the direct downwind direction averaged 2.2 colonies/cu ft (78/cu m). The highest single concentration recovered was 28 colonies/cu ft (990/cu m) at 100 ft (30.5 m) directly downwind from the tank gallery.

Sampling times during the day were not reported. Air temperature, relative humidity, wind speed, and wind direction were measured on site. Recoveries decreased with increasing air temperature above 76°F (24°C), and a combination of elevated air temperature and low relative humidity was reported to act synergistically to reduce the recovery levels more than by either variable alone. Wind speeds of 17 to 36 mph (27 to 58 km/hr) during sampling had no discernible effect on organism recoveries. Samples taken simultaneously in pairs directly and at an angle downwind from the tank gallery were used by King and King *et al.* to develop aerosol dispersion pattern predictions. However, no projections were made.

King and King *et al.* reported that a much larger number of enteric bacterial genera and species was identified from colonies recovered downwind than from those recovered upwind; they thus were confident that the aeration tanks were the true source of the recovered airborne organisms. *Staphylococcus aureus* was recovered 5 times as often downwind as upwind from the aeration gallery. This result led King and King *et al.* to suggest that primary pathogenic bacteria are dispersed into the air in the aeration process. *Alcaligenes faecalis* was recovered frequently from downwind but never from upwind samples, and its possible value as an indicator organism in studying airborne bacteria from wastewater was suggested by King and King *et al.* The bacterial concentration in the wastewater being aerated was estimated to be 1.2×10^{10} organisms/ml.

With the activated sludge seeded to a level of 10^9 *Serratia marcescens*/ml, 20 colonies of this species/60 cu ft (12/cu m) were recovered from air samples taken an unspecified distance downwind from the aeration tank. King and King *et al.* concluded that the number and the type of bacteria found in the wastewater and subsequently recovered from the air downwind pose a possible health hazard to plant employees and nearby residents.

Pereira and Benjaminson[15] assayed bacteria in air inside and downwind from an enclosed aerated wastewater tank ventilated with forced draft blowers discharging through stacks about 30 m above the ground level. From its description, the tank was a conventional activated sludge aeration tank. Recoveries per cubic meter of air sampled averaged 21,800 viable colonies inside the building, 890 inside the exhaust stack, and 48 at a sampling station 360 m downwind from the tank. Upwind control samples recovered a mean of 17 colonies/cu m of air.

All samples were taken with the Andersen six-stage impactor. Viable particles recovered inside the building were reported to be primarily in the 5- to 10-μ diam range, and those in the stack ranged from 2 to 5.5 μ in diam. Specific viable particle size data are presented only for samples taken in the stack. The geometric mean aerodynamic particle diameter of the viable aerosol in the stack was calculated from Pereira and Benjaminson's data to be 3.2 μ, with a standard deviation of 1.9.

Salmonella and *Mycobacterium* genera were recovered from the liquid wastewater being aerated; *Klebsiella* and hemolytic *Streptococcus* genera were recovered from air inside the building and at the downwind station; and *Klebsiella* and *Mycobacterium* genera were recovered from air in the exhaust stack. None of these organisms was recovered from upwind air samples. Acid-fast bacilli were recovered from glycerin-coated swabs exposed for 15 min in the air stream of the stack at 22 m above ground level. Guinea pigs inoculated with these organisms developed lesions containing acid-fast bacilli within 6 wk.

Air in the stacks was also sampled while ozone (0.3 and 0.6 ppm) was being added to the exhaust air. No differences in bacteria recovery levels could be detected, and Pereira and Benjaminson concluded that the ozone exerted no effective bactericidal action on the airborne organisms. They attributed the lower recoveries in the stack, as compared with those inside the building, to gravimetric fallout of viable particles inside the building. This may have been a factor because the location of the inside samplers was not specified and they could have been collecting unevaporated spray, particularly as a result of the fact that the reported particle size of the viable aerosol inside the building was larger than that in the stack. Pereira and Benjaminson also reported obtaining higher recoveries on sheep blood agar (SBA) than on tryptic soy agar, and they attributed this to the ability of SBA to support the growth of injured or fastidious organisms.

Pereira and Benjaminson concluded that their data indicate that airborne organisms from wastewater pose a potential health hazard, particularly for the young, elderly, and infirm residing in areas contaminated by gaseous effluents from wastewater treatment plants. No mention is made of these organisms being hazardous to plant personnel. Pereira and Benjaminson recognized the need to correlate their findings with epidemiological data.

Goff *et al.*,[16] by using the Andersen six-stage impactor, sampled the air for total bacteria and coliforms downwind from two trickling filter plants at distances from 5 m to 5 km. Simultaneous upwind samples were taken, and periodic assays were made of the bacteria concentration in the trickling filter influent.

Total bacterial concentration in the liquid wastewater ranged from 1.5×10^6 to 4.0×10^6 organisms/ml, and the coliform concentration averaged 2.1×10^4/ml. Reported airborne organism recoveries attributed to the trickling filters were

Sampling Distance Downwind from Source (m)	Mean Counts/cu ft of Air: Total Bacteria		Coliform Bacteria	
	Day	Night	Day	Night
5	14	54	7	21
50	4	13	2	5
700	12		6	
3,000			0	

These data are downwind concentrations minus the upwind concentrations. Upwind concentrations averaged 180 viable particles/cu m of air, and only 1 to 2 percent of these were coliforms. Goff *et al.* reported sampling to 5 km downwind from the trickling filter plants, but no data past 3 km are presented. The particle sizes of the aerosols were reported to be largely less than 5 μ in diam.

Goff *et al.* reported that greater concentrations of both total and coliform organisms were recovered at night than during the day and concluded that solar radiation had a detrimental effect on airborne organism survival. Goff *et al.* examined the effect of wind speed, relative humidity, and air temperature on organism recoveries and concluded that solar radiation, low relative humidity, and low wind speed significantly reduced viable aerosol emissions. They were unable to assess the effect of any single variable. For example, although greater numbers of coliform organisms were recovered at higher relative humidities, the higher relative humidities also occurred at night, so the effects of radiation and humidity could not be separated.

Goff *et al.* make no conclusions from the data regarding health hazards. They do conclude that *E. coli* may be used as an indicator of air pollution from wastewater plants and other fecal sources.

For this summary of investigations, the authors of the present study did not fully review foreign language literature, but several abstracts and brief summaries were reviewed. Wanner,[22] by using liquid impingers and a slit sampler, took samples of airborne organisms over and downwind from unspecified wastewater aeration tanks. He reported recovering 10^3 to 10^5 organisms/cu m at 1 m above the aeration tanks and between 100 and 400 organisms/cu m of air at downwind distances of 200 to 400 m. He recovered more organisms downwind at higher wind speeds and lower air temperatures than at lower wind speeds and higher air temperatures, but he did not attempt to assess the individual effects of these variables.

Woratz [23] recovered coliform organisms from the spray of brush aerators at an activated sludge plant and concluded that they presented a definite health hazard to plant employees.

Malz and Neugluck,[24] on the other hand, found no increase in airborne bacteria near open sewers and wastewater works that were not described in their abstract; they measured organism recoveries on agar settling plates exposed 50 cm above the wastewater surface.

Related laboratory and field investigations. Higgins [26] examined in the laboratory the generation rate of viable bacterial aerosols from simulated wastewater inoculated with a known variety of coliforms, *Streptococcus* sp., and *S. marcescens* and also with spores of *B. subtilis* as a reference species expected to be only slightly affected by the generation process. The aerosols were generated by pumping air through fritted glass diffusers submerged in the inoculated liquid. The aerosols discharged into a small wind tunnel, and air samples were taken downwind on agar settling plates, a slit sampler,[50] and the Andersen six-stage impactor.

Higgins reported recovery of *S. marcescens* in concentrations of up to 5 times those of *B. subtilis* under comparable conditions. By allowing for an expected loss of viability of *S. marcescens* during aerosolization, he calculated that its aerosolization rate may have been up to 20 times that of *B. subtilis*. The coliform group yielded very low recoveries. *E. coli* and *Escherichia freundii* were not recovered. *A. aerogenes* was recovered only from large unevaporated droplets, and *Escherichia aurescens* was recovered in concentrations less than 1 percent of that of *B. subtilis*.

Higgins concluded that the generation rate of viable aerosols is species selective, probably because of a difference in the cell concentration at the surface from that in the bulk of the liquid. He concluded that *S. marcescens* concentrated at the surface. He suspected, but could not demonstrate, that the low aerosol generation rate of the coliforms was a result of their migration away from the surface. On this basis, Higgins suggested that coliforms may not be a reliable index of air pollution arising from wastewater aeration and that total colony count may be a more accurate measure.

Blanchard and Syzdek [51, 52] observed the same phenomenon and found the concentration of *S. marcescens* in drops formed from bursting bubbles to be from 10 to 1,000 times that in the liquid from which the bubbles were generated. The differential factor increased with drop size and decreased with higher bacterial concentrations in the bulk liquid. In liquid containing organism concentrations comparable to those in wastewater, the concentration in the drops ranged from 1 to 100 times that of the bulk liquid.

Higgins [26] maintained isokinetic sampling conditions with the slit sampler and the Andersen impactor by the use of glass sampling probes up to 31 mm in diam inserted into the wind tunnel. These were reduced in size through a 90-deg bend and connected to a 6-mm inside diam tube, which led to the sampler inlets. He acknowledged that this arrangement probably discriminated against larger diameter particles. Higgins could not isolate from his data any effects attributable to air or liquid temperature or relative humidity variations. Neither could the number of aerosol particles generated nor their size distribution be conclusively shown to be dependent on bubble diameter.

Higgins calculated the particle size of viable particles, as assayed by the Andersen impactor, to be 2.8 to 3.1 μ in diam for aerosols from liquids containing 40 mg/l solids and 4.8 to 5.8 μ for aerosols from liquids containing 130 to 260 mg/l solids.

In his discussion of the health significance of aerosolized microorganisms, Higgins observed that wastewater may contain a sufficient number of pathogenic bacteria or viruses, particularly when epidemics or hospital wastes are involved, to pose a significant threat in the vicinity of a wastewater treatment facility. He stated that if pathogens concentrate at the wastewater surface, as does S. *marcescens*, a relatively low concentration of pathogens might constitute a significant health hazard. Higgins recommended that species-selective aerosol generation studies be extended to pathogenic bacteria and viruses.

Smith [27] examined the generation of viable aerosols from bubbles bursting at the surface of aerated liquid inoculated with 10^7 to 10^8 spores of *B. subtilis* var. *niger*/ml. He found that the aeration bubble size, the concentration of spores in the liquid, and the composition of the liquid affected viable aerosol generation. The viable particle generation rate increased in direct proportion to the spore concentration of the aerated liquid up to a concentration at which each droplet contained at least one viable spore and remained essentially constant thereafter. This point occurred at a spore concentration of 1.5×10^7/ml of test liquid for 0.5-mm diam bubbles and at 2.1×10^6 for 5.7-mm diam bubbles.

At spore concentrations comparable to bacterial concentrations in wastewater, Smith found that the aerosol generation rate ranged from 3 mil viable particles/l of aeration air with 0.5-mm diam bubbles down to approximately 5,000 viable particles/l of aeration air with 5.7-mm diam bubbles. The generation rate of viable particles was higher from liquid containing various concentrations of salts and organic chemicals than it was from demineralized water.

The maximum median particle diameter of viable aerosols generated from spores in demineralized water was found to be 5.7 μ with aeration bubbles of approximately 1-mm diam; the minimum median particle diameter was found to be 3.7 μ with 5.7-mm diam aeration bubbles.

Generally, increased solids concentrations in the aerated liquid were accompanied by increased median viable particle diameters, as assayed by the Andersen six-stage impactor. However, median viable aerosol diameters became smaller as the concentration of some salts and acids in the aerated liquid increased. In experiments in which the aerated liquid contained 400 to 1,600 mg/l solids, Smith found that the median viable aerosol particle diameter was around 5 μ but ranged from 2.4 to 8.6 μ. The diameter was thus small enough to permit lung penetration of a substantial proportion of the particles.

Smith concluded that aeration of contaminated wastes and other liquids could be responsible for the production of hazardous aerosols. He suggested that wastewater treatment processes using aeration be considered hazardous from the viewpoint of airborne infection and recommended (*a*) epidemiological studies of respiratory and other diseases in treatment plant personnel and residents near wastewater aeration facilities, (*b*) consideration of aerosol control mechanisms such as enclosure of aeration tanks and treatment of exhaust air or replacement of direct aeration with other processes, and (*c*) development of a suitable index organism for evaluating the airborne infection potential from wastewater treatment processes.

Buchan,[28] Buchan *et al.*,[29] and Buchan and King [30] postulated that protein-bearing material aerosolized from an activated sludge aeration tank could, if it were inhaled, have direct health effects in terms of allergenic response. They assayed total and protein-bearing particles aerosolized from such a tank by sampling downwind with membrane filters. Upwind controls were similarly assayed. Counts were made by means of appropriate protein-staining techniques and light field microscopy (400× magnification for protein-bearing particles and 970× for total particles).

Buchan, and Buchan *et al.* recovered

9,129 more protein-bearing particles/cu ft (322,300/cu m) downwind than upwind and determined the mean particle diameter to be 2.58 μ, with a standard deviation of 2.35 (geometric). Total particles collected downwind from the tank had mean particle diameters of 0.25 to 0.33 μ. Buchan and Buchan *et al.* calculated that the activated sludge aeration tank was emitting 3.5 × 10^6 particles/sec/sq ft of surface area (10.8 × 10^6/sec/sq m), of which 76,000/sq ft (820,000/sq m) were protein bearing. They observed that increased wind speed resulted in increased emissions but that the particle size was smaller. They found no noticeable decrease in the concentration of protein-bearing airborne particles 200 ft (61 m) downwind, the farthest distance sampled, and concluded that not only plant employees but also nearby residents were exposed to plant emissions. They recommended an epidemiological study of the respiratory ailments and allergies of those exposed to the emissions to clarify the health significance of the plant emissions.

Glaser and Ledbetter [31] sized settleable airborne particles generated by an activated sludge aeration tank. Samples were collected from air in a container floating on the tank surface. Settleable particles down to 1 μ in diam were assayed and were reported to have a mean particle diam of 13 μ. Air from the container was reported to contain 15,600 particles/cu ft (548,000/cu m) of air as measured by recovery on a membrane filter. Each of the particles was larger than 1 μ in diam.

Reviews and investigations addressing health significance. Ledbetter *et al.*[32] concluded that the added hazard of respiratory disease from working at a wastewater treatment plant is quite small and may tentatively be termed insignificant. From earlier data [9] on *Klebsiella* recovery near activated sludge aeration tanks, they calculated that an employee would inhale at most 105,600 *Klebsiella*/month. They thus considered the risk of *Klebsiella* infection minimal in light of the findings of Henry *et al.*,[53] which stated that, in squirrel monkeys, 850,000 inhaled *Klebsiella pneumoniae* caused an infection but 730,000 did not.

Ledbetter *et al.* also made a health survey, the only one found in the literature, of pneumonia incidence among 287 employees of large Texas wastewater treatment plants, using 383 employees of drinking water treatment plants as controls. The number of definite pneumonia cases was found to be the same (0.002/yr/employee) in the two groups, while the incidences of flu and colds were higher among wastewater workers by factors of 50 and 28 percent, respectively. Ledbetter *et al.* considered the findings tentative because of the number of doubtful cases and stated their intention to categorize these and to analyze the results statistically at a later date. This survey is all the more important in that it is the only one that could be found that had a direct bearing on increased disease rates associated with occupational exposure to viable aerosols in wastewater treatment operations. Most other surveys and reviews of the health of workers in the wastewater industry have been only marginally related in that they were concerned with waterborne disease transmission [33] or accident and injury rates.[34]

Sorber *et al.*,[35] Sorber,[36] and Sorber *et al.*[37] concluded that spraying of wastewater for irrigation may be a public health hazard through inhalation of aerosolized pathogenic microorganisms, particularly viruses. They observed that wastewater disinfection processes are often far less effective against pathogenic bacteria and viruses than against the usual indicator organisms and that these groups also survive longer in air than do indicator organisms.

Sorber *et al.*[37] postulated a model in which wastewater receiving various degrees of treatment might be sprayed onto fields under several meteorological conditions. They calculated that persons working at ground level 200 m downwind from the fields may inhale as many as 20 infectious units of airborne virus in 10 min and that a high probability for inhalation of significant numbers of enteric viruses would exist beyond this distance. They pointed out that sunlight, higher air temperature, and, particularly, low relative humidities would tend to reduce the viability of airborne bacteria and viruses but stated that neither these factors nor aerosol dilution by diffusion should be relied on to eliminate possible health hazards associated with such aerosols. Instead, they recommended adequate buffer zones around spray irrigation sites or adequate virus removal from wastewater before spraying. They calculated that wastewater filtration and disinfection would accomplish a reduction of 3 orders of magnitude in airborne virus concentration, while an 800-m buffer zone would provide a reduction of only 2 orders of magnitude. Sorber *et al.* concluded that removing or destroying viruses in wastewater before spraying would minimize the virus aerosol hazard.

Citing the suggestion of Dowling [54] that inhalation of low concentrations of pathogens may confer a degree of immunity, Sorber *et al.*[35] suggested that treatment plant workers and permanent populations near wastewater treatment plants may not show health effects from exposure to viable wastewater aerosols because of such immunity but that sporadic exposure of other populations may result in adverse health effects, which would be virtually untraceable by epidemiological means.

Sepp [38] made an extensive literature review of the health aspects of using wastewater for irrigation, including a summary of U. S. and foreign regulations related to wastewater spraying. He cited numerous outbreaks of disease attributed to the consumption of food irrigated with wastewater. His review indicated that laws regulating the spraying of wastewater are generally directed toward preventing the contamination of food and fodder but that aerosol dispersion from sprayed wastewater is recognized as a hazard in some regulations. Eastern European countries generally prohibit the spraying of wastewater, and some western European countries require safeguards, such as adequate surrounding buffer areas to protect waterworks and habitations and prohibition of spraying during strong winds. Some U. S. states were reported to require disinfection of wastewater before using it for crop irrigation.

In another review of wastewater spraying practices, Sepp [39] included "protection of the public from airborne spray" as a public health requirement in spraying operations. He also indicated that no spraying should be done during strong winds if the spray may be carried toward nearby human habitations.

Jensen [40] demonstrated the occurrence of tubercule bacilli in considerable numbers in the wastewater systems of several towns containing tuberculosis clinics. Citing literature tracing tuberculosis cases to the immersion of persons in contaminated water, he suggested that airborne tubercule bacilli from wastewater treatment plants could pose an infection hazard to plant workers through inhalation. He particularly singled out as sources biological (trickling) filters, activated sludge aeration tanks, spray irrigation with wastewater, and the handling of dried sludge.

In his extensive literature survey of biological aerosols as air pollutants, Finkelstein [41] listed wastewater treatment processes as one of many producers of viable aerosols; in general, he concluded that airborne transmission of human and animal diseases is essentially limited to indoor spaces and closely confined outdoor spaces. His rationale was that pathogens cannot reproduce in air and generally do not survive long because of adverse conditions of temperature, humidity, and sunlight.

Acknowledgments

Credits. This investigation was supported by the National Institute for Occupational Safety and Health Grant #5-T01-OH 00099-04 and was conducted as part of a graduate training program at the University of North Carolina, Chapel Hill.

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