

Effect of an Activated Sludge Wastewater Treatment Plant on Ambient Air Densities of Aerosols Containing Bacteria and Viruses

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Received 26 December 1984/Accepted 27 February 1985

Bacteria- and virus-containing aerosols were studied during the late summer and fall seasons in a midwestern suburb of the United States before and during the start-up and operation of an unenclosed activated sludge wastewater treatment plant. The study showed that the air in this suburban area contained low-level densities of indicator microorganisms. After the plant began operating, the densities of total aerobic bacteria-containing particles, standard plate count bacteria, total coliforms, fecal coliforms, fecal streptococci, and coliphages increased significantly in the air within the perimeter of the plant. Before plant operations, bacteria were detected from five genera, *Klebsiella*, *Enterobacter*, *Serratia*, *Salmonella*, and *Aeromonas*. During plant operations, the number of genera identified increased to 11. In addition to those genera found before plant operations, *Escherichia*, *Providencia*, *Citrobacter*, *Acinetobacter*, *Pasteurella*, and *Proteus*, were also identified. Enteric viruses were detected in low densities from the air emissions of this plant. Only standard plate count bacteria remained at significantly higher than base-line densities beyond 250 m downwind from the center of the aeration tanks. Fecal streptococci and coliphages appeared to be more stable in aerosols than the other indicator microorganisms studied. In general, the densities of microorganism-containing aerosols were higher at night than during the day. The techniques used in this study may be employed to establish microorganism-containing aerosol exposure during epidemiological investigations.

Aerosols that contain microorganisms are generated through natural processes. Such microorganism-containing aerosols occur widely in nature and are generated in the oceans by wave action (5, 6) and on the land when wind suspends decaying vegetative debris through soil erosion (24). Microorganism-containing aerosols are reportedly capable of very long-range transport (6). Some of these aerosols can contain microorganisms pathogenic to humans as well as to agricultural livestock and crops.

Many other sources of microorganism-containing aerosols are, however, generated through human activities in both urban and rural areas (8, 9). Population growth in urban areas has increased the density of domestic wastes which must be disposed of in a safe and environmentally sound manner. Consequently, expansion of existing waste treatment or utilization facilities is necessary. Some of these facilities have, however, been shown to emit microorganism-containing aerosols under certain conditions. Sewage treatment plants (1, 20), sanitary landfills and resource recovery systems (17), and compost operations (18), for example, have all been considered as potential sources of airborne infectious microorganisms. Because of economic, environmental, or political constraints, some of these facilities are located in densely populated regions of urban or suburban communities. In these cases, a determination of the contribution of the facilities to the microorganism content of the ambient air may allow an evaluation of the potential for adverse health or environmental effects.

One such facility, the O'Hare Water Reclamation Plant, located near the O'Hare International Airport in the City of Des Plaines, Ill., a suburban area northwest of Chicago, was constructed to be operated as part of the regional Metropolitan Sanitary District of Greater Chicago system. The prox-

imity of this plant to a residential area was the subject of concern for several years because of the potential for exposure of the surrounding population to microbial aerosols emitted from the plant. The purpose of this study was to determine the contribution of the newly constructed plant to the base-line microbial aerosol densities that already existed in the vicinity of that plant. The results reported here were part of a larger study of microorganism densities in air at the wastewater treatment plant site (13).

MATERIALS AND METHODS

Field sampling approach. (i) Study site and sampling locations. The O'Hare Water Reclamation Plant was designed as a 272,000 m³ (7.2 × 10⁶ gallons per day (MGD)) capacity two-stage municipal sewage plant. The dimensions of the two-stage aeration basins, including walkways, were ca. 160 by 200 m. The microbial aerosol densities reported in this paper were determined during the late summer and fall season in the vicinity of this plant before it was operated and again during a similar season after it began operating. The first sampling season was from 15 August to 7 November the year before the plant was started, and the second was from 26 August to 12 November during the first year of plant operations.

Aerosol sampling sites were located at various distances downwind (<150, 150 to 250, and >250 m) or upwind of the center of the aeration tanks. As shown in Fig. 1, this center was positioned ca. 80 m from the north and south aeration tank boundaries and ca. 100 m from the east and west boundaries of the first- and second-stage aeration tanks, respectively. This location was also ca. 120 m from the northwest and southwest corners of the second-stage aeration tanks and about the same distance from the northeast and southeast corners of the first-stage aeration tanks. The sampling sites in closest proximity to the aeration tanks were

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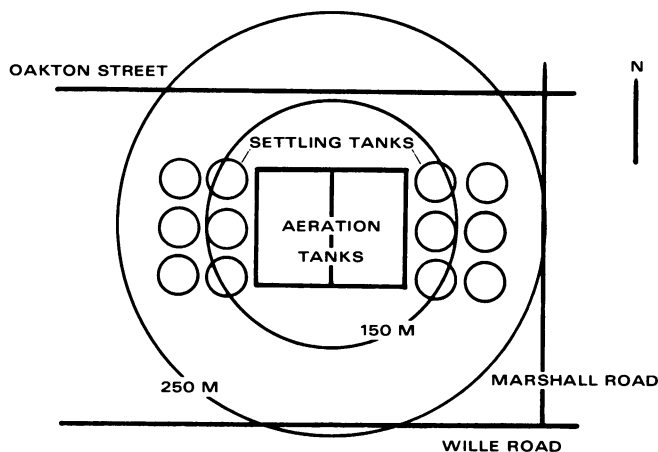


FIG. 1. O'Hare Water Reclamation Plant. Sampling distances with respect to center of aeration tanks.

located <150 m downwind from the center of the tanks. Since the exact location of these sites was determined by physical accessibility, many samples were taken near or at the aeration tank boundary.

(ii) **Air sampling methods.** Sampling instrumentation and procedures were selected with the goal of determining the density of microbial aerosols of potential public health significance. The organisms sampled, the sampling devices used, and the reporting units for the organisms studied are listed in Table 1. Air samples were taken during the day (0800 to 1959 h) and night (2000 to 0759 h) by using multistaged impactors (MSI) (3) and large-volume scrubbers (LVS) (12) at each of the four general locations with respect to the center of the aeration tanks.

Total aerobic bacteria-containing particle (TABCP) samples were taken at an air sampling rate of ca. 0.03 m³/min by using MSI samplers (Andersen, Inc.). These samplers were swabbed with 70% ethanol and loaded with six glass plates containing 27 ml of Trypticase soy agar (BBL Microbiology Systems) with 0.01 to 0.02% cycloheximide and coated with 0.2% oxyethylene docosanol green, to reduce desiccation. Samples of standard plate count (SPC) organisms, total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), coliphages (CP), and enteric viruses (EV) were taken with LVS at air sampling rates of 0.6 to 0.9 m³/min. These samplers were sterilized by conventional autoclaving before field use. The sampling fluids used were Trypticase soy broth (25%) for SPC organisms, TC, FC, and FS; dulcitol selenite broth (50%) for *Salmonella* sp.; phage assay broth (25%) for CP; and Hanks balanced salt solution with 25% nutrient broth for EV. Particle-laden sampling fluid was aseptically collected in serum-stoppered bottles. The fluid was recirculated during sampling for EV, and evaporation losses were replaced with sterile distilled water. Collected samples were maintained on wet ice during sampling and transport.

Assay and enumeration. When available, standard methods for assay and enumeration were those previously described (2). The standard membrane filter procedure was used for TC, FC, and FS assays, and the SPC procedure was used for SPC organism determinations. Selected TC colonies were identified by using API 20E strips (Analytab, Inc.) with the oxidase test.

Assays for *Salmonella* sp. and CP were performed in enrichment tubes and were enumerated by using the most-

probable-number (MPN) method for estimating the density of small numbers of microorganisms in fluids. CP were assayed on *Escherichia coli* C3000 cells as previously described (11). *Salmonella* cells were enriched in dulcitol selenite broth at 40°C for 24 h and then streaked onto xylose lysine desoxycholate agar as previously described (16). Black colonies observed after 24 h of incubation at 37°C were identified with the API system.

Samples for EV assay were sonicated (model W-375; Heat Systems-Ultrasonics, Inc.), filtered through 0.2- μ m heat-inactivated fetal calf serum-pretreated membrane filters and divided into two portions. The first portion was assayed for cytopathic effect. If this portion was negative, then the second portion was also assayed for cytopathic effect. Otherwise, the second portion was assayed by the plaque method.

EV assays were performed on Buffalo Green Monkey Kidney and WI-38 cell monolayers. Cell cultures were grown at 37°C in minimum essential medium with 10% fetal calf serum and antibiotics (gentamicin, 50 μ g/ml; amphotericin B [Fungizone], 2.5 μ g/ml). Cells were washed with Hanks balanced salt solution, inoculated with sample, and rewarmed after a 2-h virus adsorption period. The inoculated cultures were then overlaid with either liquid- or agar-based minimal essential medium and assayed as previously described (10). Virus isolates were identified by serum neutralization by using pooled enterovirus antisera.

Quality assurance. Before use and periodically throughout the project, each sampler was calibrated to determine air flow rates by using a precalibrated mass flow meter or anemometer. These samplers were numbered, and collected samples were identified with a particular instrument. Field loading and unloading of MSI was monitored by using control agar plates. LVS and MSI were autoclaved at 121°C for 15 min.

During sample assay, two negative and two positive organism control assays were performed for each field trial. For the positive controls, suitable dilutions of *Enterobacter aerogenes*, *Escherichia coli*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, MS-2 phage, and poliovirus type 1 were used for TC, FC, FS, *Klebsiella* sp., *Salmonella* sp., CP, and EV tests, respectively. Periodic bacteria assays for SPC organisms with tryptone glucose extract by the spread plate procedure on Trypticase soy agar were used to confirm the SPC technique and the TABCP medium. Plates used for TABCP determinations were incubated at 35°C for 24 h before use and discarded upon evidence of colonial growth.

To minimize the possibility of cross-contamination, positive control assays were performed after sample assays. In

TABLE 1. Microorganism-containing aerosol sampling

Microorganisms	Sampling device	Air vol sampled (m ³)	Units
TABCP	MSI	0.8-1.7	CFU
SPC	LVS	0.8-1.7	CFU
TC	LVS	5-10	CFU
FC	LVS	10-20	CFU
FS	LVS	10-20	CFU
<i>Salmonella</i> sp.	LVS	30-60	MPN VU ^a
CP	LVS	30-60	MPN PFU
EV	LVS	400-600	CPU ^b

^a VU, Viable units.

^b CPU, Cytopathogenic units.

addition, positive EV control assays were always performed in a separate laminar flow hood from that used for sample assays. Virus isolates were stored at -70°C in a locked freezer containing no other virus stocks.

Data analyses. Data were analyzed to test the hypothesis that the densities of the microorganism-containing aerosols studied were not significantly different after the O'Hare Water Reclamation Plant began operations than were observed in the ambient air at the site of the future plant. The nonparametric Mann-Whitney U test was used for determining significance at the $P < 0.01$ level (22, 23). Geometric means were weighted by p/n , where p was the number of positive observations and n was the total number of observations. This weighting procedure was similar to that described by Snedecor and Cochran (23).

RESULTS

During the late summer and fall after the plant began operating the sewage flow rates ranged from 56,100 to 183,000 m^3/day (14.8 to 48.4 MGD) during aerosol sampling. The average flow rate was 106,000 m^3/day (27.9 MGD). A total of 738 assays were performed for TABCP, SPC, TC, FC, FS, and CP organisms among the four sampling locations during the fall seasons of this study. Of the 316 assays performed before plant start-up, 29, 28, 28, and 16% were from the upwind, <150-m-downwind, 150- to 250-m-downwind, and >250-m-downwind locations, respectively. Of the 422 assays performed after the plant was started, 30, 31, 24, and 15% were from those respective locations. Of the 30 EV assays, 12 were performed before and 18 were performed after the plant was started. One-half of these assays were performed from the <150-m-downwind and the upwind locations.

SPC. The geometric mean aerosol densities of SPC bacteria are shown in Table 2. The downwind densities increased significantly during the night at all downwind sampling locations after the plant began operating. At the <150-m sampling location the increase was from 55 to 1,325 CFU/m^3 ($P < 0.001$), at the 150- to 250-m location the increase was from 65 to 410 CFU/m^3 ($P < 0.01$), and at the >250-m location the increase was from 60 to 262 CFU/m^3 ($P < 0.01$) after the plant began operating. Significant increases were not observed during the daytime at any location.

TABCP. TABCP aerosol densities (Table 2) were generally lower than those observed for the SPC bacteria during plant operation, suggesting that aerosolized particles

TABLE 2. Aerosol densities of SPC bacteria and TABCP

Microorganisms	Density (CFU/m^3)							
	Upwind		Downwind (m)					
			<150		150-250		>250	
	Day	Night	Day	Night	Day	Night	Day	Night
SPC bacteria								
Preoperation	43	26	49	55	32	65	52	60
Postoperation	157	297	220	1,325	102	410	194	262
TABCP								
Preoperation	141	76	65	102	113	104	163	270
Postoperation	79	52	272	373	115	175	194	191

contained multiple organisms. After plant operations commenced, significant geometric mean density increases ($P < 0.001$) were observed during both the day (65 to 272 CFU/m^3) and night (102 to 373 CFU/m^3) at the <150-m-downwind location, but were not observed at further downwind locations. The upwind and most distant downwind locations were sometimes (depending on the prevailing wind direction) downwind from heavy road traffic, which contributed dust to the area and could have increased the density of TABCP aerosols at those sites, especially during daytime rush-hour periods.

TC. TC geometric mean densities (Table 3) increased significantly ($P < 0.01$) from 0.27 to 5.17 CFU/m^3 at the <150-m location during the night after the plant began operations. During the day, these densities increased from 0.24 to 6.81 CFU/m^3 . These differences were substantial but were not accepted as significant ($P = 0.026$). The increases of from 0.18 to 0.57 CFU/m^3 during the night and from 0.28 to 0.86 CFU/m^3 during the day at the 150- to 250-m-downwind locations were not significant. The densities beyond 250 m downwind and at upwind locations did not increase significantly during either the day or night after plant operations started.

FC. No FC-containing aerosols were detected during either the day or night before the plant began operating. As shown in Table 3, the densities of these bacteria increased at all downwind locations after the plant began operating. During plant operations, the weighted FC aerosol densities were 0.01 CFU/m^3 upwind of the plant during both the day and night. At night the geometric mean densities were 2.09,

TABLE 3. Aerosol densities of TCFC, and FS

Microorganisms	Density (CFU/m^3)							
	Upwind		Downwind (m)					
			<150		150-250		>250	
	Day	Night	Day	Night	Day	Night	Day	Night
TC								
Preoperation	0.21	0.28	0.24	0.27	0.28	0.18	0.22	0.12
Postoperation	0.22	0.09	6.81	5.17	0.86	0.57	0.40	0.34
FC								
Preoperation	<0.04	<0.06	<0.03	<0.06	<0.04	<0.06	<0.03	<0.06
Postoperation	0.01	0.01	1.67	2.09	0.18	0.64	0.29	0.15
FS								
Preoperation	0.13	0.88	0.04	0.70	0.14	1.00	0.06	0.58
Postoperation	0.04	0.83	0.29	2.07	0.15	1.21	0.48	0.86

TABLE 4. Aerosol density of CP

CP	Density (MPN PFU/m ³)			
	Upwind	Downwind (m)		
		<150	150-250	>250
Preoperation	$<2.5 \times 10^{-2}$	5.0×10^{-2}	$<2.6 \times 10^{-2}$	2.8×10^{-2}
Postoperation	1.2×10^{-2}	7.3×10^{-2}	4.9×10^{-2}	7.6×10^{-2}

0.64, and 0.15 CFU/m³ at the <150-, 150- to 250-, and >250-m-downwind locations, respectively. Daytime densities at the <150- and 150- to 250-m-downwind locations (1.67 and 0.18 CFU/m³, respectively) were lower than those observed at night. At the most distant downwind locations, the density was 0.29 and 0.15 CFU/m³ during the day and night, respectively.

FS. FS densities were consistently higher at night than during the day at all sampling locations both before and during plant operations. Night densities before the plant began operating were higher than those observed during the day during plant operations at all sampling locations. Background FS geometric mean densities (Table 3) ranged from 0.04 to 0.14 CFU/m³ during the day and from 0.58 to 1.00 CFU/m³ during the night at all downwind locations. After the plant began operations, night densities ranged from 0.86 to 2.07 CFU/m³ at the >250- and <150-m-downwind locations, respectively.

Members of the family Enterobacteriaceae. The diversity of bacterial isolates identified from TC aerosols increased after the plant began operations. Before plant operations, bacteria were detected from five genera: *Klebsiella*, *Enterobacter*, *Serratia*, *Salmonella*, and *Aeromonas*. During plant operations, the number of genera identified increased to 11. In addition to those genera found before plant operations, the genera *Escherichia*, *Providencia*, *Citrobacter*, *Acinetobacter*, *Pasteurella*, and *Proteus* were also identified.

Before plant operations, the genus *Enterobacter* constituted 80% or more of the bacteria identified from each of the four sampling locations. *Enterobacter agglomerans* was the most frequently identified species at all locations. Other species were identified with extremely low frequency. For example, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, and *Klebsiella oxytoca* were each detected with low frequency (on two or fewer occasions) before the plant began operations.

During plant operations, the most frequently isolated genera were *Escherichia* followed by *Enterobacter*, *Klebsiella*, and *Citrobacter*. Most of these isolates were found in aerosols <150 m downwind of the center of the aeration tanks. A total of 88% of the *Klebsiella*, 96% of the *Escherichia*, and 92% of the *Enterobacter* isolates were identified at downwind locations closer than 250 m. The most frequently isolated *Klebsiella* species was *K. pneumoniae*, followed by *K. oxytoca*.

Salmonella sp. Before plant operations, one isolate, confirmed as *Salmonella cholerae* was detected within 150 m downwind of the center of the future aeration tanks. During plant operations, *Salmonella* sp.-containing aerosols were detected on one occasion at <150 m, and on another occasion *Salmonella paratyphi* was identified in samples at 150 to 250 m downwind of the center of the aeration tanks.

CP. The CP aerosol density increased at all sampling locations during plant operations. As shown in Table 4, the CP geometric mean density increased significantly ($P < 0.01$) from 5.0×10^{-3} MPN PFU/m³ to 7.3×10^{-2} MPN

PFU/m³ at the <150-m-downwind location after the plant began operating. The CP density was also significantly higher at 150 to 250 m downwind after as compared with before starting plant operations. CP densities were highest at the >250-m-downwind locations both before and after starting plant operations at 2.8×10^{-2} and 7.6×10^{-2} MPN PFU/m³. The background density after plant operations began was increased by the high CP density that was observed in the single positive sample. Nevertheless, significant differences could not be accepted using the data analyses procedures employed in this study.

EV. No EV were detected before the initiation of plant operations in air sample volumes ranging up to 552 m³. After the initiation of plant operations, EV were detected on two of nine occasions. No viruses were detected during the daytime in air sample volumes of up to 428 m³. The two occasions on which EV were detected were during the night within 150 m of the operating aeration tanks. The general wind direction on both occasions was from the south at ca. 4 to 5 m/s. These viruses were detected in Buffalo Green Monkey Kidney but not in WI-38 cells from air sample volumes of 211 and 193 m³, respectively. When detected, the EV density ranged from 4.7×10^{-3} to 1.0×10^{-2} CPU/m³ of air. Although one virus isolate was not identified with the Lim-Benyesh Melnick antisera pool, the two remaining viruses were both identified as coxsackievirus B-1.

Aerosol particle size. The size of bacteria-containing aerosols was greater after the plant began operating compared to pre-operations. Although the aerodynamic count median diameter of TABCP was generally in the 3.3- to 4.7- μ m range, the percentage of the particles counted that was below 3.3 μ m decreased at all downwind sampling locations after the plant operations began. The cumulative percentage of these aerosols decreased from 38 to 24, 40 to 26, and 45 to 26 at downwind locations of <150, 150 to 250, and >250 m, respectively. The percentage of aerosols in this smaller size range remained relatively unchanged in the samples taken at the upwind locations.

DISCUSSION

The air near residential environments is not sterile but contains microorganisms, some of potential enteric origin, which have unknown significance to human health and welfare. The air of the suburban environment of a major U.S. midwestern metropolitan area, for example, contained microorganisms of potential enteric origin before initiation of operations at a major wastewater treatment plant site. Densities of these microorganisms in the air were generally highest during the night. Of the indicator bacteria studied during the late summer and early fall season before plant operations, FS observed in the night had the highest densities, with geometric means ranging from 0.58 to 1.00 CFU/m³. TC ranged from 0.12 to 0.28 CFU/m³, but no FC were detected in air sampling volumes of 10 to 20 m³. Enteric bacteria from five genera, *Klebsiella*, *Enterobacter*, *Serratia*, *Salmonella*, and *Aeromonas*, were identified. The geometric mean densities of TABCP ranged from 65 to 163 CFU/m³ during the day and from 76 to 270 CFU/m³ during the night over four different sampling locations. SPC bacteria densities were lower, ranging from 32 to 51 CFU/m³ during the day and 29 to 65 CFU/m³ at night. The densities of the TABCP aerosols were within the same order of magnitude as those observed in the suburbs of an eastern metropolitan area, which had a geometric mean of 79 CFU/m³ (15).

The sources of microorganism-containing aerosols are multiple and may include natural processes as well as those produced by human activities. One such activity, that of wastewater treatment, increases the aerosol density of certain microorganisms above those observed in the absence of such a plant. After starting the operation of a wastewater treatment plant, the greatest increases in the densities of SPC, TABCP, TC, FC, FS, CP, and EV were observed at downwind locations closest (<150 m) to center of the aeration tanks. Many of these samples were within a few meters of the edge of the tanks. The densities of all of the microorganisms studied decreased at 150- to 250-m-downwind sampling locations. Although these densities remained above the pre-plant-operation levels, significant ($P < 0.01$) increases at locations beyond 250 m downwind were only observed for the SPC bacteria.

The occurrence of potentially infectious microbial aerosols per se does not provide evidence of associated health risks. Several studies on the health of populations living near wastewater treatment processes did not demonstrate significant adverse health effects due to exposure to wastewater-generated microorganism-containing aerosols (20). These studies, however, all had major limitations that made it difficult to attach significance to either the positive or negative findings. There were low numbers of persons subject to exposure to high doses of microbial aerosols, and the rate of this exposure throughout the population could not be adequately and quantitatively determined (14). Furthermore, the epidemiology of exposure to microbial aerosols in heterogeneous and mobile communities is largely affected by the secondary exposure rate and by the susceptibility of the population.

The possibility of exposure to higher densities of microbial aerosols in a suburban environment are greater during the night than during the day. These higher densities observed during the night indicate either increased microorganism survival rates or greater atmospheric stability, or both. The data clearly demonstrate that daytime sampling of the environment for microorganism-containing aerosols will result in underestimation of the densities to which a population may be exposed at night. Since the highest ambient airborne microorganism densities occur at night, any efforts to determine maximum exposure rates or to limit or control exposure will be most effective when employed during that time period.

Higher densities of SPC bacteria than of TABCP at downwind locations during plant operations demonstrate the differences of assaying bacteria and bacteria-containing particles. If the particles contain multiple bacteria, enumeration for SPC bacteria will promote disassociation of these organisms. The data suggest that more bacteria were associated with airborne particles during plant operations than before such operations began.

This study documents the first reported isolations of enterovirus-containing aerosols from outdoor secondary wastewater treatment processes. EV were, however, only detected at the <150-m-downwind sampling locations and only at night. Other investigators have reported low-level enterovirus densities around spray irrigation facilities (19, 25) and in enclosed secondary treatment processes (21). Earlier studies demonstrated that the aerosol density of EV around wastewater treatment processes is very low compared with indicator bacteria and CP and that detection requires sampling methods with high sensitivities (10, 12).

Both CP (10) and FS (7) have been suggested as possible indicators of the potential for microbial aerosol contamina-

tion from wastewater treatment processes. CP, which increased at all downwind locations after plant start-up, have been shown to be more stable than coliform bacteria at distant locations from the source (10). The CP, f2, was also demonstrated to be more stable than bacteria during treatment with chlorination and were detectable at distances up to 137 m downwind (4). These CP have also been demonstrated to be more stable in wastewater aerosols than are polioviruses (K. F. Fannin, S. C. Vana, and R. Ehrlich, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1981, Q115, p. 219), suggesting that they may survive in aerosols to travel to greater distances than certain EV.

The data from the present study indicate that CP are stable in aerosols, with the highest densities observed at distances of >250 m downwind from an operating wastewater treatment plant. The FS also demonstrated greater stability than the other indicator bacteria at these downwind locations, but showed marked differences in densities between day and night. Although these data support the conclusions that certain FS and CP organisms could be used as indicators of domestic wastewater treatment plant aerosols, the relatively high background densities of FS, especially at night, increase the difficulty in determining the originating source of these bacteria. Whereas the sensitive detection of appropriate indicator organisms do not indicate densities of pathogens, it can measure the potential for airborne contamination and could serve as a measure of exposure for epidemiological studies.

ACKNOWLEDGMENTS

The cooperation of the City of Des Plaines, Ill., the Metropolitan Sanitary District of Greater Chicago, and the Institute of Gas Technology is gratefully acknowledged. The programming assistance of Wladyslaw Toczycki, the encouragement of James Fenters, and the technical assistance of Paul Andressen, Charles Gradle, William Mega, and Dennis Sullivan are appreciated. Although the research described in this article has been funded in part by the U.S. Environmental Protection Agency through grant R-806062 to the IIT Research Institute, it has not been subjected to the Agency's review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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