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**A FIELD TECHNIQUE MEASURING VIRUS DECAY AND
POTENTIAL AEROSOL HAZARD FROM WASTEWATER
SPRINKLER IRRIGATION**

by

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ABSTRACT

The increased use of domestic wastewater for irrigation purposes has stimulated a growing practice of sprinkler irrigating from oxidation ponds and other domestic wastewaters. Aerosols generated from these sprinkler irrigation systems may contain potentially hazardous pathogens. Subsequently, the aerosols can contain infective viruses which can be carried through the air to surrounding populations. Thus, a public health hazard can be created by sprinkler irrigating domestic wastewater.

This study is an investigation of a means by which the virus decay rate of viruses in aerosols and the potential hazard of sprinkler irrigation aerosol clouds may be examined.

A means of injection of a human and animal virus simulant, MS-2 bacteriophage, is described. In addition, the factors which are known to effect the survival of viruses in aerosols are discussed. The ambient air factors that are known to effect virus survival and which are discussed include relative humidity, air temperature, solar radiation, and aerosol age. The suspending fluid factors that are known to effect virus survival and which are discussed include dissolved inorganic salt content, dissolved organic content, filterable solids and pH.

The decay rate of the aerosolized MS-2 was measured with an all-glass impinger (AGI-30) when the wind velocity and distance of the sampler from the sprinkler system, and the initial and final concentrations of aerosolized MS-2 virus were known.

The aerosol hazard of a domestic wastewater sprinkler irrigation system is defined in terms of the likelihood of infective aerosol particles to be inhaled and penetrate the human lung. Thus, the aerosol hazard is a function of aerosol particle size. The aerosol particle size distribution of the infective aerosol cloud was measured with the Andersen sampler.

A high and homogeneous concentration of the virus in the wastewater was insured by using pressure differentials in the sprinkler irrigation delivery line. By injecting the MS-2 virus into the line at a constant rate along with a tracer, *Bacillus subtilis* var *niger* (*Bacillus globigii*) spores, the decay rate of the virus during airborne exposure to environmental factors could be determined. The decay rate was determined assuming the environmental factors had no affect on the concentration of the tracer.

It was proposed that the environmental engineer, after knowing the virus decay rates under varying environmental conditions, can define buffer zones which would be required around sprinkler irrigation sites. The buffer zone would reduce the possibility of contaminating humans by prohibiting access.

The usefulness of the field technique was demonstrated and the MS-2 was found to undergo a 33.3 percent decay per minute in the dark (no solar radiation) at 33 percent relative humidity, 70.6 percent dissolved organic material, 29.4 percent dissolved inorganic salts, 30.1 mg/l filterable solids, and 17^o C air temperature. The aerosol cloud resulting from the spray irrigation process appeared potentially hazardous because the Andersen sampler collected 84.2 percent of the virus infective droplets in the size range that could be inhaled.

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Nature of Problem

In recent years, the practice of using sprinkler irrigation for disposing of domestic wastewater on agricultural land has become increasingly popular. This practice is partially the result of Public Law 92-500 which will prohibit the discharge of pollutants into navigable waters by 1983. In addition, the practice is also a result of water supply shortage in agricultural areas and the practice can provide moisture and nutrients to the soil and crops.

Sprinkler irrigation of domestic wastewater, although beneficial in some respects, is not without its disadvantages. Aerosols generated from sprinkler irrigation systems using domestic wastewater may contain pathogenic microorganisms including viruses. Consequently, the aerosols containing viable viruses could be carried to populations downwind from spray irrigation sites and become a potential health hazard.

Many factors have been studied independently which were found to contribute to airborne virus decay. For the environmental engineer to reduce the possible public health hazard of virus infective aerosols, a technique has been developed for field use to measure the factors which affect virus decay and determine the potential aerosol hazard of sprinkler irrigation systems.

Objectives

The objectives of this study were to develop a technique:

1. That would optimize the reliability of data collected under field conditions where sprinkler irrigation plots are located.
2. To measure and define the decay rates of virus laden aerosols under varying environmental stresses.
3. To measure the possible hazard of the virus laden aerosols which are emitted from a sprinkler irrigation system.
4. To relate the data obtained from the decay rate to data obtained from measuring possible aerosol hazard.

Significance of Study

The development of the technique is significant for various reasons. Until this time, research directed toward the study of virus decay in infective aerosols has taken place only under laboratory conditions. Under these conditions, a single environmental stress was varied while holding other environmental stresses constant. This technique, developed for field use however, measures all the stresses as they exist at the time of sampling. This is useful because the virus decay rates, as they exist in the field under varying conditions, can be defined. After recording the measurements over the range of values found in ambient air conditions, statistical correlations can be calculated to determine the stresses most likely to contribute to virus infective aerosol decay rates. The stresses may be synergistic or antagonistic to each other in relation to virus decay rates and this information, when known, is significant because it can be gainfully used to predict the conditions for optimum sprinkler irrigation.

The technique provides a means for controlling the homogeneous concentration of virus in the spray irrigant. The spray irrigant is the suspending fluid for the virus and will be referred to as the suspending fluid. The homogeneous virus concentration in the suspending fluid is important because it contributes to an aerosol which, when measured, is a representative sample of the aerosol cloud.

The use of the technique can provide valuable information relating the potential hazard of an aerosol cloud to its affect on public health and the use of the technique during different environmental conditions can determine the best conditions for decreasing potential aerosol hazard. The techniques, when used, can also be useful in understanding conditions which can be used to enhance virus infective aerosol inactivation and to define the need and extent of buffer zones around domestic wastewater sprinkler irrigation sites. The buffer zones can be defined in terms of the probability that a certain percentage of the virus would decay in the zone and where public access would be prohibited.

CHAPTER II

REVIEW OF THE LITERATURE

Viruses in Wastewater

It has been reported in the literature that man is responsible for excreting more than 100 kinds of viruses. Concentrations as high as 1.1×10^4 plaque forming units (PFU) of virus per liter have been found in raw sewage (Berg, 1973, p. 87). The significance of viruses in wastewater resides not only in numbers but also in virus infectivity. In studies by Katz and Plotkin (1967) it was reported that "the smallest quantities of viruses that can be detected in susceptible cells in culture, our most sensitive indicators of infection, are sufficient to produce infection in man" (Berg, 1973, p. 87). Shuval (1970) reported from a broad study that recoveries of enteric viruses ranged from 5 PFU/l to more than 11,000 PFU/l in a city in Israel. Average recoveries per community in this study ranged from about 500 PFU per liter to more than 1,600 per liter.

The persistence of relatively high quantities of viruses in sewage may contribute to a public health hazard. Although sewage is an unfavorable environment to viruses, Berg (1973, p. 88) reported that viruses can be detected in a primary effluent containing 3 mg/l of chlorine. It was reported (Berg, 1973, p. 91) that oxidation ponds and trickling filter field tests appeared erratic for detecting the presence of viruses, but it was conclusive that the biological treatment often removed little or no viruses.

Significance of Sprinkler Irrigation

The persistence of large quantities of viruses in domestic wastewater is particularly significant to populations which are directly exposed to domestic wastewater sprinkler irrigation. This is becoming especially true since sprinkler irrigation is becoming an increasingly popular method of domestic wastewater disposal. Hence, the production and transfer of infective aerosols to populations downwind from sprinkler irrigation sites should be a major public health concern.

The primary concern with infective aerosols is related to aerosol particle size. Infective aerosol

particles in the 2 - 5 μm diameter range are thought to be a particular threat to the gastro-intestinal tract. Aerosol particles in this size range are normally deposited on the upper respiratory tract where they are removed by ciliary action and eventually are swallowed. Enteric viruses found in this aerosol size range are a potential hazard to the public health (Adams and Spendlove, 1970). However, higher incidence of infection is likely to occur when aerosol particles in the 1 - 2 μm diameter range are inhaled. These aerosols are transported to the alveoli of the lung where they are deposited by settling (Spendlove, 1975). Respiratory viruses found in this aerosol size range are a particular threat to public health and are able to cause infection (Adams and Spendlove, 1970, p. 1220).

Factors Which Affect Aerosol Hazard

The aerosols, as they are carried through the environment from the sprinkler site to lung penetration site, are exposed to many environmental factors which are detrimental to the virus laden aerosol. The discussion of the detrimental environmental factors which follows will enhance the understanding of methods which can be used to maximize virus decay rates and minimize the aerosol hazard.

Nozzle spacing

It has been reported that "the spacing and type of nozzles used will significantly influence the aerosol particle size formation from ... spray distribution systems. Spray nozzles are manufactured which operate over a pressure range from 5 - 130 psi with increased pressure generally proportional to increase in aerosol formation" (Sorber and Guter, 1971, p. 2). Increase in aerosol formation refers to an increase in the number of droplets in the small size range.

Aerosol transport

The direction of aerosol movement is dependent on the aerosols mass as well as some physical factors such as gravity and wind velocity.

Particles reach a terminal velocity when frictional forces of the air and gravitational forces are equal. Stokes' law describes the terminal velocity by

$$V = \frac{\rho d^2 g}{18\eta}$$

in which

- ρ = particle density
- d = particle diameter
- g = acceleration due to gravity
- η = viscosity of air

Below a particle size diameter of 10 μm , Stokes' law becomes inaccurate because "the particle diameter begins to approach the mean free path of gaseous molecules" (Dimmick, 1969a, p. 4) and the particles tend to fall faster than Stokes' law predicts. "The correction (usually referred to as Cunningham's correction) may be approximated and used in the equation by adding 0.08 μm to the diameter if we are calculating velocity. The rate of fall of the 1 μm particle, calculated by this simple correction, is 3.48×10^{-3} cm/sec" (Dimmick, 1969a, p. 4-5). If gravity were the only factor influencing the vertical movement, it would take 9.7 hours for the particle to fall 4 feet.

Other forces besides gravity obviously influence the vertical movement of aerosols when they are subjected to field conditions. It is possible then, for an infective particle of 1 μm to impact before 10 hours after being sprayed 4 feet into the air. It is not important in this paper to define the factors which influence the movement of aerosols but rather to emphasize that some physical factors may be used which would influence the movement of virus infective particles. For example, turbulent air streams, found under normal ambient air conditions, impart a velocity to particles across the lines of air flow and cause the particles to impact on surfaces. It may be feasible to increase the probability of impaction before lung penetration by surrounding the irrigation site with trees and spray the irrigant during optimal wind conditions for maximum virus decay.

Aerosol size change

The influence of evaporation on the aerosol contributes to a reduction in particle size. A droplet formed and too large for inhalation, may undergo size reduction. Holding other environmental factors constant, the decreasing droplet sizes will increase the number of infective viruses per droplet, the number of infective droplets in a volume of air, and the incidence of infection by lung penetration. However, "if the liquid to be dispersed contains dissolved solids, particles will not completely evaporate, but will be reduced to a new size" (Dimmick, 1969b, p. 27). An equation for evaporation reported by Dimmick (1969b, p. 27-28) is

$$\bar{d}_f = \bar{d}_o \sqrt[3]{s \rho_o / 100\rho}$$

in which

- s = gm. of solute/100 gm. solution
- ρ_o = density of solute
- \bar{d}_o = average diameter, initial
- \bar{d}_f = average diameter, final

Problems of supersaturation, surface tension, internal particle pressure, selective adsorption, hygroscopicity and selective crystallization—all should influence the final (droplet) size; and all (of the factors as mentioned) interfere with the possibility of using a simple equilibrium calculation such as Raoult's law to arrive at a more theoretically satisfying answer (to final size) (Dimmick, 1969b, p. 27-28).

Domestic sewage, which obviously contains gases and organic and inorganic dissolved and suspended solids, makes a mathematical model of the resulting particle size a nebulous subject. However, solids in domestic wastewater can be used advantageously to decrease the potential aerosol hazard of an infective aerosol cloud. The presence of algae cells, for example, in oxidation pond effluents can limit the extent of aerosol particle size reduction and prevent the possibility of particle inhalation.

Factors Which Influence Airborne Virus Decay

Species

The species of virus determines to some extent, how well the virus will survive environmental stresses. The virus structure, although simple in framework, varies significantly enough among species to change the response of the virus to the environmental stresses. A typical virus contains either ribonucleic or deoxyribonucleic acid surrounded by a capsid constructed of a symmetric protein shell. The nucleic acid and capsid together is referred to as the nucleocapsid. The virion is the complete infective virus particle which may be the nucleocapsid or, in more complex virions, include a surrounding envelope (Jawetz, Melnick, and Adelberg, 1970, p. 276) which may contain lipid material. The virus' morphology and size differs among the virus groups. Virus morphology is defined by the symmetry of the virus structure, presence of a membrane, and the presence or absence of a tail structure. The viruses differ among groups in that their structures contain different compounds which differentiate them according to susceptibility to physical and chemical agents. A virus not containing lipids is resistant to ether or chloroform which are lipid solvents. Lipid containing viruses are generally more resistant to drying than non-lipid containing viruses and more resistant to some of the environmental stresses.

Viruses contain no enzymes which could enable them to metabolize their own constituents. Therefore, they must infect a specific living host cell which will replicate the virus structure by the host cell's metabolism.

Physical and chemical factors in the domestic wastewater and in the air are in dynamic change. The response of the virus to the changes differs among the species and inactivation may be the result of a single mechanism or combination of mechanisms which confront the virus with unfavorable conditions.

Suspending fluid

The composition of the fluid in which the viruses are suspended before and during airborne flight has a marked effect on the survival of virus infective aerosol droplets. A review of the literature indicates three important suspending fluid components which influence the survival and detection of virus infective aerosols.

1. *Dissolved inorganic salt content.* The importance of dissolved inorganic salts on the survival of viruses in the airborne state was observed in studies by Benbough (1971).

Benbough's inability to obtain reproducible results from repeated airborne virus studies was attributed to slight variations of the dissolved inorganic salts in the suspending fluid.

Benbough (1971) found that the Langat virus, a flavivirus of the togavirus group (previously classified as a group B arbovirus), when aerosolized in tissue culture fluid, evinced a mid-range sensitivity to relative humidity in the presence of salts. The sensitivity was reduced when the salts were removed from the suspending fluid. The sensitivity to relative humidity was shifted to a high sensitivity when organics were removed and the salt concentration maintained. He also found that when the virus was sprayed

...from 5 percent (w/v) solutions of NaCl or KCl, virus infectivity was rapidly inactivated at intermediate relative humidities but not at higher or lower relative humidities: The rates of inactivation were greater for NaCl than for KCl. When virus was sprayed from a 5 percent (w/v) solution of LiCl the inactivation rate was not affected at very high relative humidities. At lower relative humidities the inactivation rate increased, so that below 50 percent no infective virus could be recovered in aerosols within 1 minute after spraying. (Benbough, 1971, p. 212-213.)

The problem of understanding a virus's response to relative humidity under the influence of inorganic salts becomes more complex when Ben-

bough's description of the responsive properties of different viruses to relative humidity is studied. When he exposed Semliki Forest virus, an alphavirus of the togavirus group (previously classified as a group A arbovirus), and Langat virus to the same inorganic salts, the Semliki Forest virus showed less sensitivity to salt concentrations than did the Langat virus.

In the same study, he found that poliovirus and T-coliphage were more sensitive to lower relative humidity than the togaviruses.

Benbough (1971) concluded from his studies that inactivation must be related to the partition of bound and unbound water between the virus, other constituents of the aerosol particle containing the virus, and the surrounding atmosphere. He found that certain solutes have contrasting effects on inactivation in aerosols of different classes of viruses. These effects were seen where some monovalent chlorides lead to togavirus inactivation but to protection of T-phages and poliovirus. He hypothesized that the inactivation of togavirus sprayed from fluids containing salts could be due to the dissolution of the lipoproteins present in the virus structure. The lipoprotein breakdown in the airborne viruses may be caused by Cl^- ions displacing bound water in membrane systems.

2. *Dissolved organic content.* The effect of dissolved inorganic salts on virus laden aerosols is changed in the presence of dissolved organic compounds. In a study, Webb, Bather, and Hodges (1963) directed some of their experiments "to test the hypothesis that compounds (are) able to protect airborne cells ... by virtue of their ability to replace structural water molecules in cell nucleoproteins." (Webb, Bather, and Hodges, 1963, p. 95.) They found that when they sprayed Rous sarcoma virus (RSV) in concentrations of inositol (an organic compound) from 0 percent to 6 percent, the recovery of virus at 30 percent relative humidity increased from less than 1 percent to 90 percent respectively. In Figure 1 from Webb, Bather, and Hodges (1963, p. 90) it appears that inositol effectively prevented the inactivation of RSV in aerosols held at 30 percent relative humidity and was non-toxic at high relative humidity levels.

The citrate buffer, containing inorganic salts has the opposite effect on airborne virus survival in that it enhances virus decay. This would be expected after studying the work of Benbough (1971) with dissolved inorganic salts.

Studies by Dubovi and Akers (1970) represented in Figure 2 reveal that MS-2 follows the same patterns of inactivation reviewed by Benbough (1971) and Webb, Bather, and Hodges (1963) concerning dissolved solids in the suspending fluid.

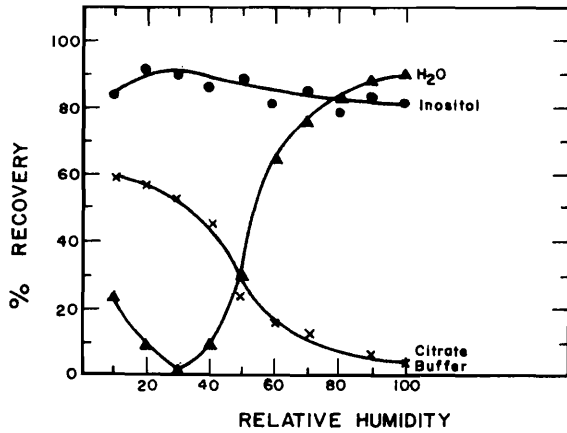


Figure 1. The effect of relative humidity and chemicals on the 5-hour survival of Rous sarcoma virus in an aerosol. (From Webb, Bather, and Hodges, 1963, Figure 3.)

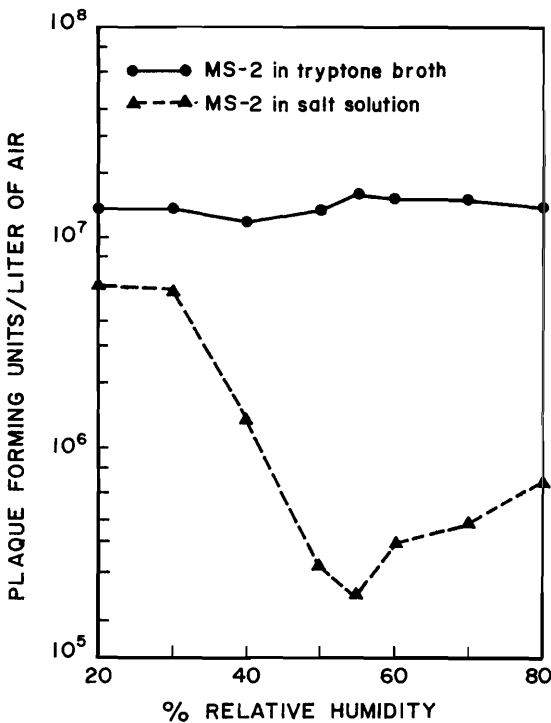


Figure 2. Recovery of MS-2 virus from 5-sec. aerosols at different relative humidity values. (From Dubovi and Akers, 1970, Figure 3.)

3. *Filterable solids.* The presence of material such as clay, bacteria and/or algal cells, and soil in the suspending fluid makes quantitative virus studies difficult to interpret. Viruses in suspending fluids with pH values close to neutrality are negatively charged. In the presence of dissolved inorganic salts, the virus is adsorbed onto surfaces (Bitton, 1975, p. 473).

Adsorption of the virus is influenced significantly by the presence of cations in the suspending fluid. Monovalent cations were found to be required in concentrations 10 times higher than the same concentrations of divalent cations (Puck, Garen, and Cline, 1951). The sorption of some viruses (polio and T₂-phage) was found to depend on the type and concentration of cations in the water (Carlson et al., 1968).

It appears from the literature that, while the cations enhance adsorption of virus to solids, dissolved organic substances in natural water compete for adsorption sites on activated carbon and can restrict the number of viruses contained on a particle (Bitton, 1975).

It was found by Tolmach and Puck (1952) that positively charged amino groups of the T₂-phage interact with negatively charged carboxyl groups of its host. The attachment of the phage to its host is suppressed at pH values which inhibit the ionization of carboxyl and amino groups.

From this discussion on solids, it is seen that the adsorption of viruses onto biological and non-biological surfaces depends in part upon the inorganic and organic composition and the pH of the suspending fluid. Adsorption also involves specific chemical groups on the surface of the virus and the solid. The pH, cations, and organic compounds can influence the attachment of viruses to suspended particles and can lead to inaccurate quantification of viruses. It can also be seen that the attachment of viruses to large solid particles can reduce the probability of viruses found in the lower aerosol size range.

As the virus infective laden fluid is exposed to the ambient air in aerosol form, other factors besides the suspending fluid become significant to the viruses ability to resist inactivation. Some of the environmental stresses known to cause virus infective aerosol inactivation are discussed below.

Relative humidity and temperature

Watkins et al. (1963) conducted a study on the survival of airborne vesicular stomatitis virus (VSV). Their results, displayed in Figure 3, show synergistic effect of relative humidity and temperature.

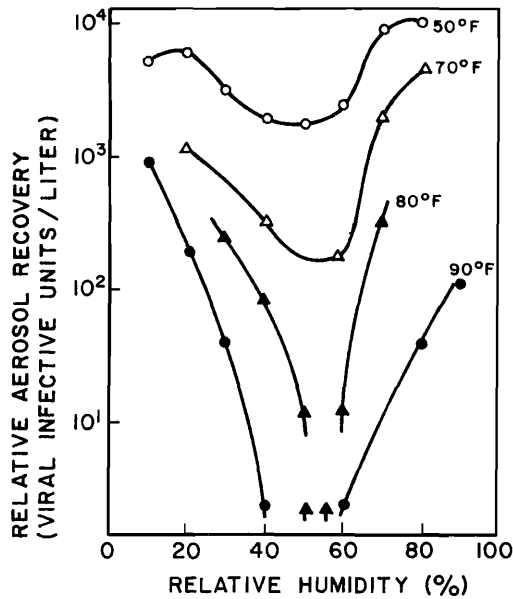


Figure 3. Effect of temperature and relative humidity on vesicular stomatitis virus aerosols aged 5 to 6 hr. From Watkins et al., 1963, p. 386.

Minimal stability of the airborne vesicular stomatitis virus (VSV) lying in the mid-range relative humidity, was significantly affected by the temperature change. The rate of aerosol decay was increased with increased temperature while the inactivation response to the humidity remained in the same range.

When Harper (1961) studied the airborne stability of vaccinia, influenza, and Venezuelan equine encephalitis (VEE) viruses, he found they survive better at 7°-12°C than at temperatures of 21°-24°C or 32°-34°C.

Songer (1967) studied the airborne stability of four viruses and found that Newcastle disease virus (NDV) and VSV survived best at 10 percent relative humidity and infectious bovine rhinotracheitis (IBR) and a T-phage survived best at 90 percent relative humidity. At 10 percent relative humidity, the NDV survived as well at 23°C as it did at 37°C. At higher humidities, lower temperature enhanced the survival of NDV.

Akers (1969, p. 311) concludes the "humidity dependent inactivation of airborne viruses is established immediately after atomization and; once established, does not drastically change with the aging of the aerosol. The effect of temperature is apparently secondary." He based this conclusion from studies on

the effects of relative humidity and temperature on the virus encephalomyocarditis (EMC) (Akers, Bond, and Goldberg, 1966) and from some of the studies previously mentioned. An initial inactivation occurs immediately after aerosolization and is not significantly temperature dependent. He explains that as the virus survives the process of aerosolization and dehydration, there is a strong reflection of temperature dependent decay.

Akers, Bond, and Goldberg (1966) reported on the survival of three strains of the Columbian SK group of viruses. The results of this study show that inactivation curves for this virus at 26°C are similar to inactivation curves from studies on the viruses, VSV, and Rous sarcoma (Webb, Bather, and Hodges, 1963), measles virus (DeJong and Winkler, 1964), polio virus (Harper, 1965), T-3 coliphage (Ehrlich, Miller, and Idoine, 1964) and VEE (Harper, 1961). The recovery of these viruses in the airborne state depends on relative humidity. Recovery is highest in the low relative humidity range and inactivation is highest in the 40 percent to 60 percent relative humidity range.

The MS-2 phage also demonstrates mid-range relative humidity sensitivity, and for this reason can be used as a model to simulate the response of many human viruses to relative humidity. The phage is a virus whose natural host is *Escherichia coli* C-3000. Because the host cell for this virus is a bacterium, it appears unlikely that it can cause human infection from lung penetration.

Dubovi and Akers (1970) reported that they had selected MS-2 for studies on airborne stability for many reasons. The MS-2, unlike many phage, does not possess a tail structure. The tail structure, necessary for some phage to infect its host cell, can be damaged and its infectivity lost during airborne flight and collection. Human viruses are not known to possess a tail structure.

Similar to many human viruses, the MS-2 is apparently icosahedral in symmetry and contains ribonucleic acid. The MS-2 can also be grown easily in high titers (10^{12} to 10^{13} PFU/ml) and the expense of growing virus in tissue culture is alleviated with the use of the bacterial host, *E. coli* C-3000.

In contrast to most airborne virus studies, Webb, Bather, and Hodges (1963) found the pigeon pox virus displayed a similar pattern to that of a vaccinia virus in that both viruses were stable and unaffected by relative humidity changes. Figure 4 demonstrates that differences in response of two viruses to relative humidity and illustrates that some viruses are not significantly affected by relative humidity.

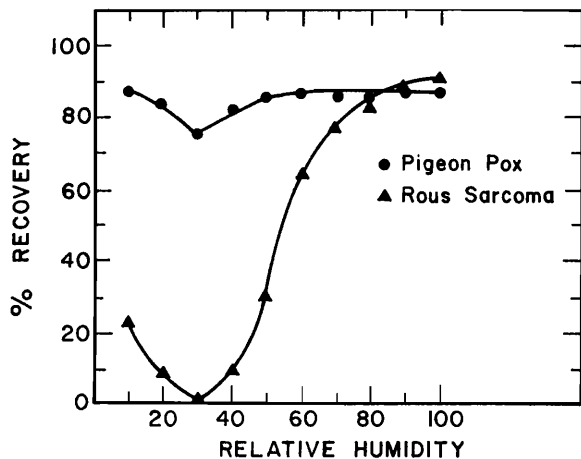


Figure 4. The effect of relative humidity on the survival of pigeon pox and Rous sarcoma viruses during 5 hours of storage in aerosols. (From Webb, Bather, and Hodges, 1963, Figure 2.)

Solar radiation

The effect of solar radiation on the activity of airborne viruses and its synergistic effect with other environmental stresses has not been studied extensively. However, there is indication that the effects of sunlight on field generated infective aerosols can be extremely detrimental to airborne virus survival.

Webb, Bather, and Hodges (1963) hypothesized that inactivation following ultraviolet irradiation "is due to rupture of hydrogen bonds between water molecules themselves or between water molecules and cellular nucleoproteins. Virus inactivation was attributed to irreversible change in the structure of the nucleic acid moiety due to loss of water molecules." (Akers, 1973, p. 79).

Berendt and Dorsey (1971) studied the effect of solar radiation on VEE exposed for one hour at different relative humidity values. They found, with no irradiation, survival was almost 90 percent and 78 percent at 30 percent relative humidity and 60 percent relative humidity respectively. After exposure to simulated solar radiation of 584 mcal/cm²/minute for 1 hour, survival was 0.02 percent and 0.006 percent at the same relative humidity values respectively. They also reported that VEE, when exposed to solar radiation for 30 minutes in the presence of 1.0 mg/ml of sodium fluorescein in the suspending medium, could not be detected at either relative humidity value. In non-irradiated suspensions of the same solution, the dye was somewhat toxic to the virus (31 percent survived after 60 minutes exposure at 60 percent relative humidity). The study indicates the devastating effect of sunlight on airborne viruses

and reveals sunlight's potential contribution in protecting the public's health from virus laden aerosols.

Figure 5 summarizes the studies of Berendt and Dorsey (1971) with sunlight.

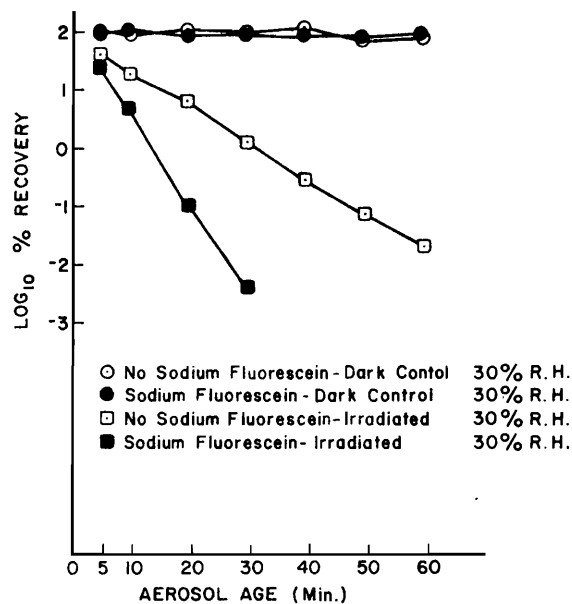


Figure 5. Effect of sodium fluorescein and irradiation on the biological recovery of VEE virus from aerosols at 30 percent relative humidity. (From Berendt and Dorsey, 1971, Figure 1.)

Gases

Ehrlich and Miller (1972) questioned the effects of pollution gases on airborne virus infective aerosols. They reported:

...the threshold NO₂ concentration (< 5 ppm) at which the inhibitory effects on the airborne VEE virus were first apparent in our studies was markedly higher than that usually found in polluted urban atmospheres. Nevertheless, this threshold level could be expected to be significantly lowered as a result of the combined effects of temperature, humidity, solar radiation, and other pollutants. (Ehrlich and Miller, 1972, p. 483-484).

Exposure time

The combined effects of the environmental stresses on the virus laden aerosol are enhanced as

the aerosol is exposed in time to the stresses. Figure 6 shows that the greater age of the aerosol significantly affects the decay of airborne viruses (Benbough, 1971, p. 212).

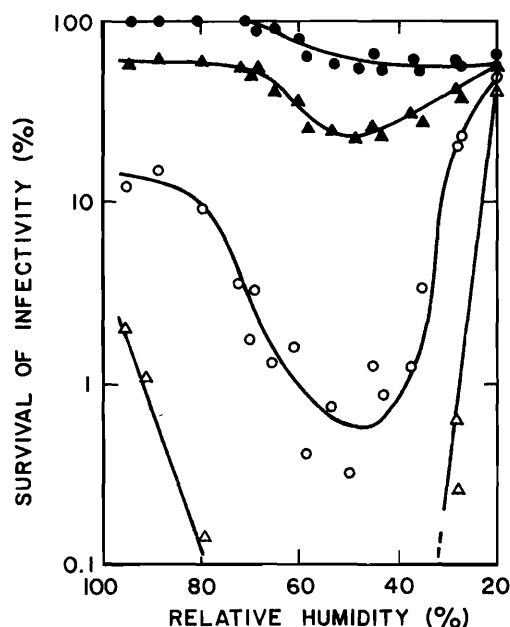


Figure 6. The influence of relative humidity on the survival of different ages of Langkat virus aerosols sprayed from culture fluid. Aerosol age: ●, 1 sec.; ▲, 5 min.; ○, 1 hr.; △, 3 hr. (From Benbough, 1971, Figure 1.)

The exposure time is influenced by many factors including the lapse rate of the atmosphere and the wind's velocity moving an infective aerosol. These variables can be eliminated for sampling purposes by sampling during times of pre-dusk and pre-dawn and during pre-frontal weather conditions when wind velocity and direction are relatively constant.

In summary, the literature indicates that there are many variables which can contribute to virus decay. Further literature review will indicate some equipment which can be used to measure virus decay and virus laden aerosol size distribution.

Collection Equipment for Infective Aerosols

All glass impinger

An all glass impinger (AGI-30) can be used to collect virus laden aerosols. The results from using it can be used to make quantitative studies of the viruses collected and define virus decay rates.

It was first suggested at the First International Symposium on Aerobiology in 1963 that "data obtained with any specialized sampler should be correlated with at least some results obtained with the AGI-30 reference sampler." (Akers and Won, 1969, p. 62). The AGI-30 allows optimum impingement when operated at an airflow rate of 12.5 liter per min. (0.44 ft³/min) and has a clearance of 30 mm between orifice and sampler bottom. It was also suggested at the meeting that the sampling medium, duration of sampling, volume of medium, collection temperature and holding time and temperature between the sampling and the assay be reported with the results.

The air sample in the AGI-30 is impinged into a collecting medium which is not likely to be harmful to the integrity of the virus or tracer.

When the ratio of pressure across the capillary to inlet pressure (1 atm) is 0.5 atm or less, particles in the aerosol impinge into the fluid at sonic velocity, the flow rate is constant, and after the sampler has been calibrated no flow meter is needed.... The air flow rate depends on the pressure ratio; if the ratio is greater than 0.5 atm, recovery efficiency of the sampler is affected and the flow rate must be measured.... For maximum collection of bacterial cells a volume of 20 ml of sampling fluid and a distance of 30 mm between the capillary tip and the bottom of the AGI-30 has been experimentally established. (Akers and Won, 1969, p. 62-63.)

Andersen sampler

The Andersen sampler was designed to measure the number and size distribution of infective aerosol droplets. Andersen (1958) presented a graphical depiction of aerosol size in relation to lung penetration. The Andersen sampler separates airborne droplets into aerosol size ranges through a cascading effect by impinging the different size droplets onto six different stages in series. Each stage is perforated with 400 holes which are positioned precisely above a glass or aluminum petri dish containing agar culture medium. The apparatus is calculated to separate droplet sizes using 27 ml of agar contained in each petri dish and located beneath each perforated stage.

Air is drawn through the device at the rate of 1 cubic foot per minute (cfm) and a jet of air from each of these holes plays on the surface of the medium. The size of the holes is constant for each stage, but is smaller in each succeeding stage, consequently, the jet velocity is uniform in each stage but increases in each succeeding stage. When the velocity imparted to a particle is sufficiently great, its inertia will overcome the aerodynamic drag and the particle will leave the turning stream of air and be impinged on the surface of the medium; otherwise the particle remains in the stream of air and proceeds to the next stage. (Andersen, 1958, p. 472.)

Sampler use

The Andersen sampler and AGI-30 function simultaneously during the same time period as close to each other as possible.

The number of infective virus droplets collected may be determined by counting the plaques on the surface of each petri dish from the Andersen sampler. Each plaque represents an infective aerosol droplet and each droplet may contain several viruses. The plaques:

...on plates 1 and 2 are scattered over the plates and should be counted in the usual manner, except when the plates are heavily loaded, in which case counting may be done through a dissecting type microscope before the colonies merge. A number of field or segments of the plate are counted, and the total number for the plate is calculated. (Andersen, 1958, p. 473.)

The use of plastic petri dishes is not recommended because, in tests, they exhibited consistently lower counts than did glass and aluminum petri dishes.

Andersen sampler characteristics

Figure 7 presented by Andersen (1958, p. 479) displays the aerosol size range that each stage can be expected to collect and describes the hazardous and non-hazardous droplet size ranges for lung penetration in relation to the droplet size.

May (1963), in his studies on the Andersen sampler found some discrepancies in the sampling characteristics of the first two stages and therefore valid calibration of the diameter characteristics of these two stages was impossible. However, his calibrations do indicate that the mass median diameter for stages 3, 4, 5, and 6 of the Andersen sampler was 4.4 μm , 3.0 μm , 1.5 μm , and 0.9 μm , respectively. These stages are the stages most important for the lung penetration description of infective droplets.

The calibration data presented by Andersen (1958) in Figure 7 can be used to determine a characteristic diameter for stages 1 and 2. The diameter of particles found on these two stages will be defined as the diameter which 50 percent of the particles are of a specified diameter and is found by dividing the diameter sampling ranges of the respective stages by 2. The characteristic diameters of stages 1 and 2 would be 12.15 μm and 7.75 μm , respectively.

The infective droplets counted on each stage are not the exact number of droplets that may have impinged on the stage. This is explained simply by the fact that there is a probability that more than one particle may have been impacted through the same hole in the Andersen sampler. Andersen (1958, p. 474) presented a table which is called the positive hole conversion table and accounts for the mentioned probability of hole duplication. He explains that the correction values in the data table were calculated by a formula from Feller (1950, p. 175).

$$P_r = N \left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \dots + \frac{1}{N-r+1} \right]$$

Where P_r is the expected number of viable particles to produce r positive holes and N is the total number of holes per stage (400). The above formula assumes that the flow of particles stops at the instant a particle enters the r th hole. Since, in the actual case of sampling, the flow of particles stops at random, the expected number of particles present if r positive holes are observed, would be equal to or greater than P_r but less than P_{r+1} and the average would be $(P_r + P_{r+1})/2$ (sic). This correction has been applied in the construction of the table. (Andersen, 1958, p. 475)

It is believed by this author after following Andersen's logic, the formula should read:

$$P_{r \text{ ave}} = (P_r + P_{r+1})/2$$

A personal communication with Andersen revealed that the final draft of the 1958 publication

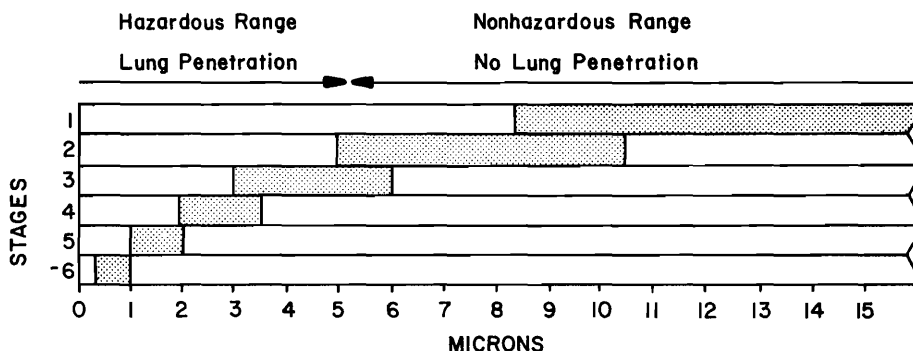


Figure 7. Relationship of stage distribution to particle size for smooth, spherical particles of unit density collected in the Andersen sampler. Each bar includes 95 percent or more of the particles collected on that stage. (From Andersen, 1958, Figure 5.)

may have been mis-interpreted or contained a typographical error.

Sampler limitations: All glass impinger and Andersen sampler

The fact that the first two stages of the Andersen sampler do not properly collect larger particles indicates that the sampler function is less than ideal.

The AGI-30 also has limitations which make its function less than completely efficient.

The potential loss of viability incurred as a result of the sampling step (AGI-30) is difficult to assess and may not always be constant. An example of variability inherent in liquid impingement samplers is that long intervals required to sample dilute aerosols (less than 600 colony-forming units per liter of air), especially at low relative humidity (RH), cause excessive cooling and evaporation of the collecting fluid that may lead to death of the organisms being assayed. (Akers and Won, 1969, p. 62)

The AGI-30 is also inefficient because

...particles of less than 0.3μ (m) may be carried by the high velocity of the jet air stream through the impinger fluid without being trapped, or particles of greater than 3μ (m) diameter might be retained by the capillary intake tube and not be impinged. (Akers and Won, 1969, p. 63)

The magnitude of sampling discrepancy between the two samplers may be examined in a study by Andersen (1958, p. 481). It reveals that an all glass impinger is consistently less efficient in collection than the Andersen sampler when bacteria were sampled.

In summary, the literature describes some equipment which may be used to measure virus laden aerosol decay rates and potential aerosol hazard. In addition, the literature indicates the variables which must be measured and which can contribute to virus decay in infective aerosols and to infective droplet sizes. Figure 8 is the data sheet which contains entry space for the variables which must be recorded during and after analysis of a sampling period.

DATE _____

COMMENTS

SAMPLE NUMBER _____

DURATION OF SAMPLE _____ TIME _____

BAROMETRIC PRESSURE _____

RELATIVE HUMIDITY DRY _____ °F _____ °C
 WET _____ °F

LIGHT INTENSITY _____ mcal/cm²/min

WIND VELOCITY _____

SOURCE STRENGTH	C _{V1} total virus	C _{T1} total tracer
10 ⁻⁵		
10 ⁻⁷		
10 ⁻⁹		
10 ⁻¹¹		
10 ⁻¹³		

SOURCE SOLIDS

CONTROLS

Aliquot (ml)	SOLIDS	FINAL	INITIAL	DIFF	mg/l
	Filtered				
	TDS organ + inorgan				
	Combust. Organ.				
	Fixed Inorganic				

- Source Tracer
- Source Virus
- Ambient Air
- Positive Virus
- Positive Tracer
- Bottom Agar Virus
- Top Agar Virus
- Host Virus
- Impinger Virus
- Agar Tracer
- Dil. Water Virus
- Dil. Water Tracer

COLLECTION

Source pH _____

AGI-30 plate dilution	C _{V1} virus	C _{T1} tracer
10 ⁻¹		
10 ⁻²		
10 ⁻³		
10 ⁻⁴		
10 ⁻⁵		

ANDERSEN SAMPLER (PFU'S/STAGE)

STAGE	ADJUSTED COUNT - P _{sn}
n	
1	
2	
3	
4	
5	
6	

Figure 8. Raw data sheet.

CHAPTER III

METHODS

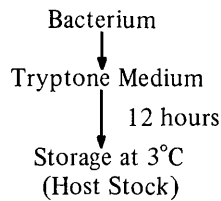
Growth, Analytical Methods, and Field Measurements

Work flow

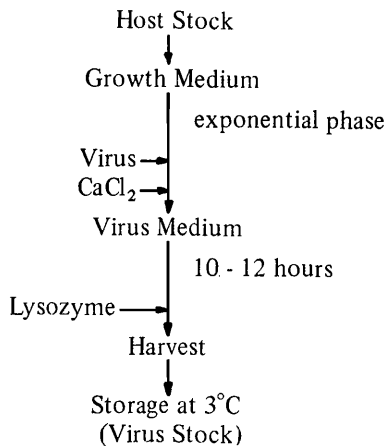
The methods used to grow, harvest, detect, and quantify the virus MS-2 and tracer *Bacillus globigii* are presented. Diagrams of the work flow are presented to simplify an understanding of the flow schemes.

Work Flow Diagrams

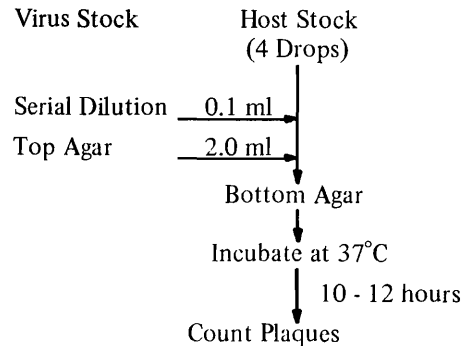
Host growth.



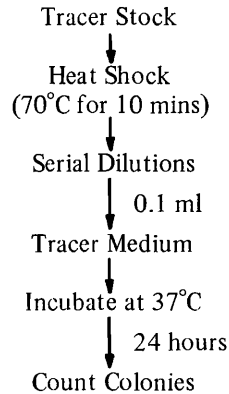
Virus growth.



Virus assay.



Tracer assay.



The virus MS-2 and its host, *E. coli* C-3000 were obtained from Thomas G. Akers of the Oakland Naval Biological Laboratory, Oakland, California. The tracer was obtained from J. Clifton Spendlove of the Dugway Proving Ground, Dugway, Utah.

Methods used for virus harvest and assay, and host growth are those of Dubovi and Akers (1970) and Tessman (1965). The assay method for tracer assay was obtained from J. Clifton Spendlove, Dugway, Utah.

Work flow discussion

1. *Host growth.* The bacterium host cell, *E. coli* C-3000 was grown in sterile tryptone broth which consisted of 13 g of tryptone and 7 g of NaCl, in one l of water. Air was constantly supplied by bubbling filtered air through the host-broth solution.

The growth cycle of the host was determined by periodically measuring the concentration of the host in time against the turbidity of the culture. When the host was grown subsequently, a measure of turbidity revealed the phase of the growth cycle.

The stock culture was prepared by growing the host at 37° C for 12 hours in the sterile tryptone broth and was then stored at 3° C.

2. *Virus growth.* The host, growing in log phase, was infected with MS-2 virus in the tryptone broth. Concurrently with the addition of MS-2, 2.5 ml of 1 M CaCl₂/l was added to promote virus adsorption to its host cell.

After 4 to 5 hours incubation at 37° C, lysozyme (0.05 mg/ml) and 5 x 10⁻³ M ethylenediaminetetraacetic acid (EDTA) was added followed by virus harvest. Air was continually supplied to the culture until harvesting began. Harvesting consisted of stirring the solution for about 30 seconds every 10 minutes. During the interim between stirring the suspension was maintained at 37° C. After 30 minutes, the suspension was centrifuged to rid the solution of cellular debris. The supernatant fluid was decanted after centrifuging and contained about 10¹² PFU/ml. This solution was the virus stock and was stored at 3° C.

3. *Virus assay.* The presence of virus in solutions was detected by the plaque method, modified from a technique described by an unpublished teaching manual of the Biology Department, Utah State University.

Four drops of host stock were added to petri dishes containing about 20 ml of sterile bottom agar. The bottom agar consisted of 12 g agar, 10 g tryptone, 5 g yeast extract and 975 ml water. After autoclaving and cooling, to about 45° C, 25 ml of 50 percent w/v sterile glucose and 2.5 ml of sterile 1 M CaCl₂ were added. Serially diluted virus solution was added to the host suspension on the bottom agar in 0.1 ml aliquots. The virus was diluted in sterile distilled water.

Two milliliters of top agar were added to the host virus puddle and mixed over the surface of the bottom agar. The plates were immediately placed inverted in a 37° C incubator after the top agar solidified. It has been reported that delaying incuba-

tion by 0.5 hour showed reduced plaque numbers (Dubovi and Akers, 1970, p. 624). It is important that assays be made on media of the same age.

The top agar consisted of sterile one percent nutrient agar and was added to the top agar when it was about 40° C.

The plaques were counted after 10-12 hours incubation. The plates can be stored at 3° C and counted later if necessary.

4. *Tracer assay.* The presence of the tracer, *Bacillus subtilis* var *niger* (*Bacillus globigii*) spores, was detected in collecting medium and suspending fluid with a tracer medium.

The tracer medium consisted of 27.5 g agar, 10.0 g dextrose, 5.0 g NaCl, 20.0 g tryptone, 0.03 g potassium tellurite and one l water, adjusted to pH of 7 and autoclaved 15 minutes. About 20 ml of the sterile medium was poured per petri dish.

A portion of the solution containing the tracer was heat shocked for 10 minutes at 70° C, then serially diluted in sterile distilled water.

A 0.1 ml aliquot from each dilution was added to the tracer medium about 24 hours after the medium was poured. The aliquot was then dispersed over the agar surface with a sterile glass rod.

The plates were inverted and incubated at 37° C for 24 hours before the tracer colonies were counted. The colonies are an orangish-brown color and slightly raised in the middle. The plates can be stored at 3° C and counted later if necessary.

5. *Tracer growth.* The spores of *B. globigii* were obtained in a concentrated form. The technique used to grow and concentrate the spores was obtained from the Dugway Proving Ground and is included in Figure 9.

Serial dilution

Figure 10 is a schematic diagram of the serial dilution technique and is included to aid in describing the dilution of the various components.

Collecting medium

When the virus and tracer were collected in the AGI-30, the collecting medium was 20 ml of double strength tryptone medium consisting of 26 g tryptone in one liter water.

The petri dishes used in the Andersen sampler were specially designed and must contain exactly 27 ml of sterile bottom agar. After the sampling period,

Bacillus Globigii FROZEN PASTE

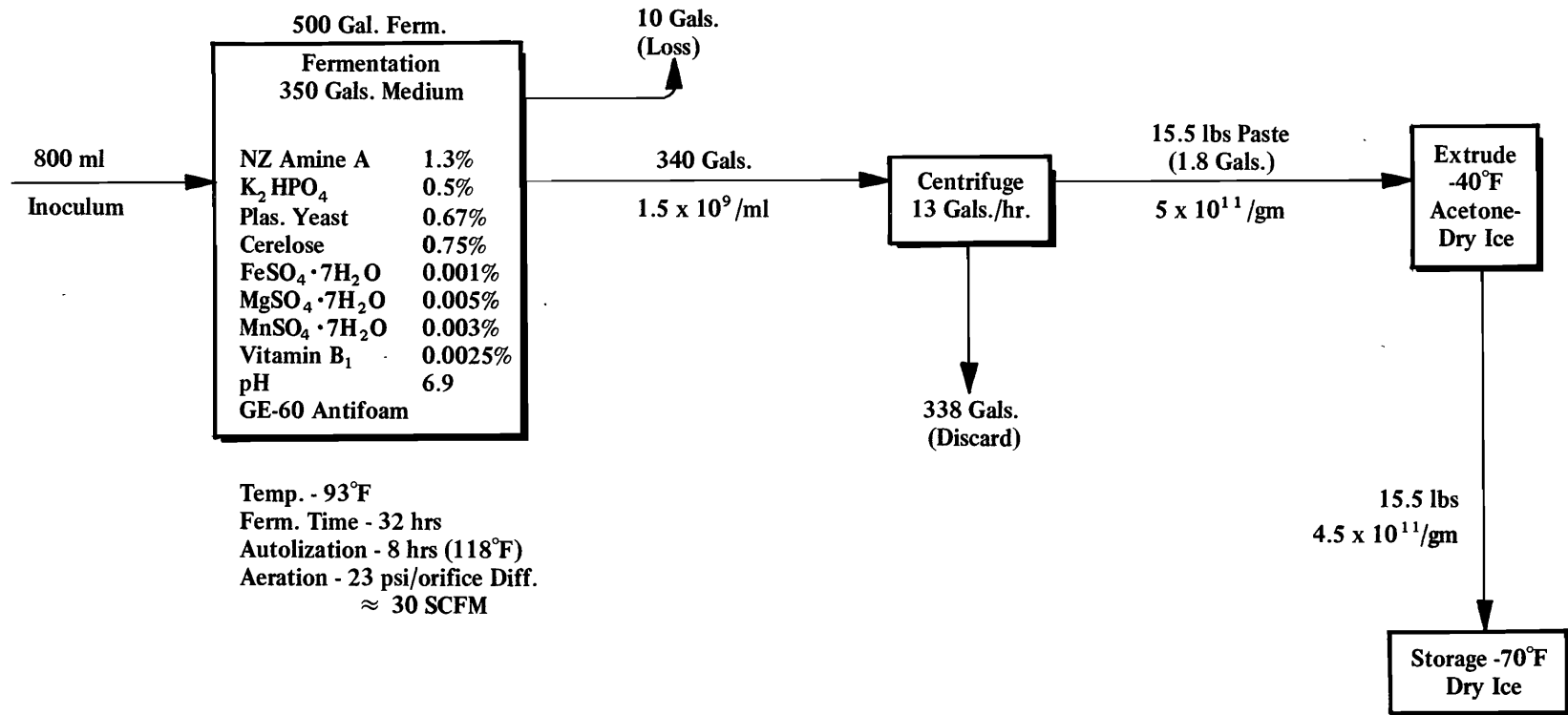
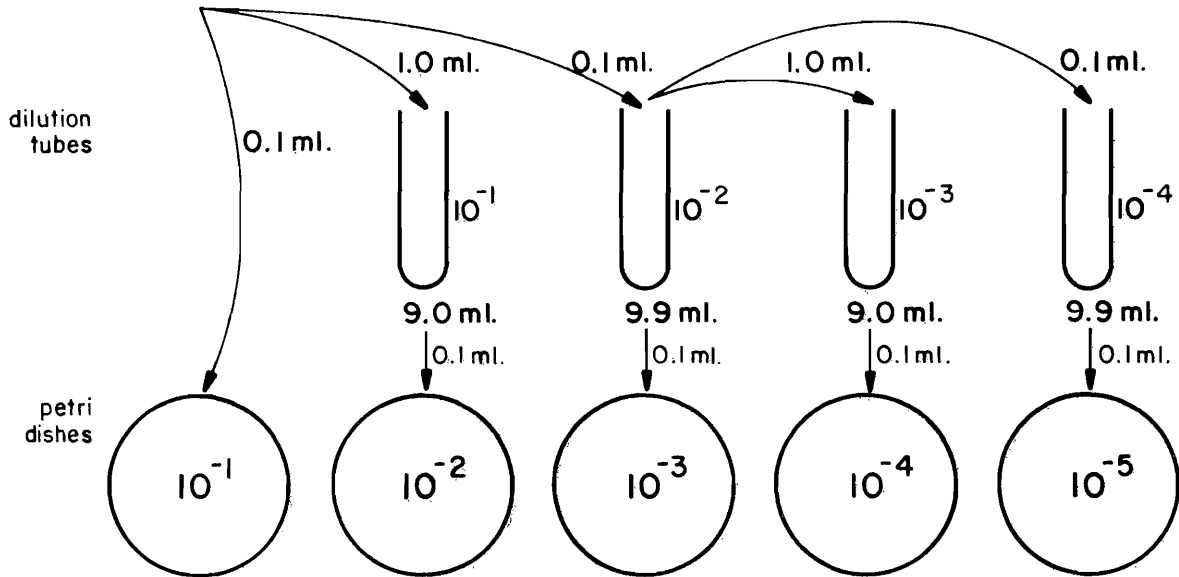


Figure 9. Growth and concentration of tracer spores.

AGI-30 dilution for virus and tracer



Source dilution for virus and tracer

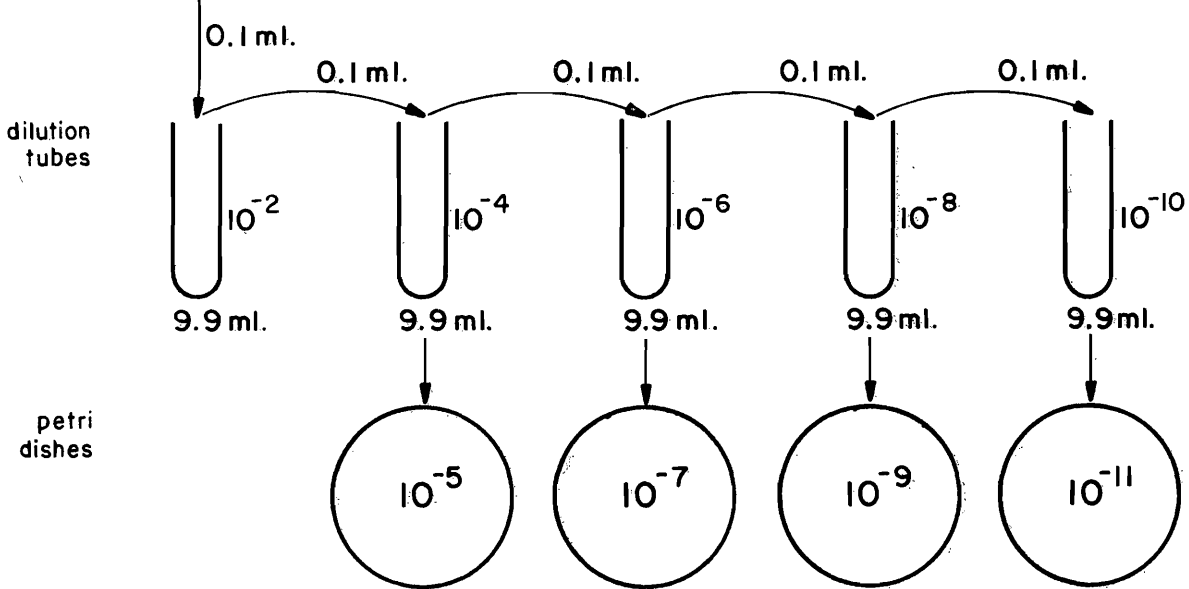


Figure 10. Serial dilution schematic diagrams.

4 drops of host stock were added to the bottom agar surface. Two milliliters of sterile top agar, at about 40°C, were placed on the surface of the bottom agar and swirled to mix the host cells over the surface of the bottom agar.

The plates were inverted after the top agar solidified, and incubated at 37°C for 10 to 12 hours. The plaques were counted after this time or stored at 3°C and counted later.

Controls

To insure the presence or absence of virus and tracer in the medium, water and air before sampling, various controls are necessary. The following is a description of each control that is included with every sample run.

1. Source tracer—a sample 0.1 ml of the suspending fluid (wastewater) is plated on a tracer media petri dish to insure there is no tracer present before the tracer is injected.
2. Source virus—a sample (0.1 ml) of the irrigant is plated on a bottom agar media petri dish and covered with 2.0 ml of top agar mixed with 4 drops of host cells.
3. Ambient air—a sample of the ambient air is sampled through the AGI-30 and 0.1 ml of the collecting medium is plated on bottom agar media and covered with 2.0 ml of top agar mixed with 4 drops of host cells. An additional 0.1 ml of heat shocked collecting medium is plated on tracer medium. The control insures the absence of virus and tracer in the ambient air. The impinger was wrapped with aluminum foil to prevent possible inactivation by light after the virus was collected.
4. Positive virus—a 0.1 ml sample of the virus solution is mixed with 4 drops of host suspension on bottom agar medium. The puddle is mixed with 2.0 ml of top agar. The control insures the presence of virus in the injection medium.
5. Positive tracer—a 0.1 ml sample of the tracer spores, after being heat shocked is plated on tracer medium. The control insures the presence of virus in the injection medium.
6. Bottom agar virus—2 drops of host suspension are placed on the bottom agar medium and spread evenly over the media's surface with a sterile glass rod. The control insures the absence of virus in the bottom agar.
7. Top agar virus—2 drops of host cell are placed on the solid surface of 2.0 ml of top agar which has been poured over the

bottom agar. The control insures the absence of virus in the top agar.

8. Host virus—4 drops of host suspension are placed on bottom agar and mixed over the surface by adding 2.0 ml of top agar. The control insures the absence of virus from the 12 hour host suspension.
9. Impinger virus—0.1 ml of the collecting medium is placed in a puddle of 4 drops of host suspension on bottom agar. The puddle is covered and mixed with 2.0 ml of top agar. The control insures the absence of virus in the impinger broth.
10. Agar tracer—a petri dish containing tracer media is used as a control to insure the absence of tracer in the sterile media.
11. Dilution water virus—0.1 ml of dilution water is placed in pool of 4 drops of host suspension on bottom agar and covered with 2.0 ml of top agar. The control insures absence of virus in the dilution water.
12. Dilution water tracer—0.1 ml of dilution water is placed on a tracer medium petri dish and spread with a sterile glass rod.

All controls are inverted after plating and incubated at 37°C. The virus controls are removed after 10-12 hours of incubation and the tracer controls are removed from incubation after 24 hours. The control plates are checked immediately after incubation to insure the presence or absence of tracer and virus.

Suspending fluid analysis

The solid constituents of the suspending fluid were determined by measuring filtered solids and determining the fraction of inorganic salts by calculating the difference between total dissolved solids and the combustible solids. The combustible solids fraction represents the dissolved organic material in the suspending fluid. Their determination is included in the following discussion.

1. Filtered solids were determined by filtering the suspending fluid through a pre-weighed Whatman, GF/C, glass fiber paper, then through a preweighed Millipore, type GS, 0.22 μ pore size filter. After filtering, the filters were oven dried at 103°C for at least 12 hours, brought to room temperature in a desiccator, and weighed. The difference in initial and final weights is the weight of filterable solids in the irrigant and is reported in mg/l. The filterable solids represents the concentration of suspended solids in the suspending fluid.
2. Total dissolved solids (TDS) of the suspending fluid were determined by placing

an aliquot of the suspending fluid in a porcelain crucible which has been pre-heated to 600°C for 15-20 minutes, cooled to room temperature in a desiccator and pre-weighed. The aliquot was dried for about 12 hours at 103°C and weighed after cooling the crucible at room temperature in a desiccator. The difference in weights is reported in mg/l and represents the total organic material and inorganic dissolved solids in the suspending fluid.

3. Combustible solids of the suspending fluid were determined by transferring the porcelain crucible, after the final TDS weighing, to an electric muffle furnace at 600°C for 4 hours. After removing the crucible and transferring it to a desiccator to cool to room temperature, a final weight was recorded. The difference between the final weight and final TDS weight is the weight of the combustible dissolved solids in the suspending fluid. The difference in weights represents what will be defined as the dissolved organic solids in the suspending fluid. It is reported as mg/l.
4. Fixed solids of the suspending fluid were determined by the difference between the TDS weight and combustible solids weight. The difference represents what is defined as the dissolved inorganic solids in the suspending fluid and is reported as mg/l.
5. It may be useful to compare the dissolved organic and inorganic solids by percentage of each. The percent dissolved organic material is found by the following formula:

$$\frac{\text{combustible wt (mg/l)}}{\text{TDS wt (mg/l)}} \times 100$$

6. Likewise the percent dissolved inorganic solids are found by the following formula:

$$\frac{\text{fixed wt (mg/l)}}{\text{TDS wt (mg/l)}} \times 100$$

7. pH—the pH of the suspending fluid can be measured with any pH instrument which will give accuracy to one decimal point.

If laboratory facilities permit, measure of the calcium, magnesium and sodium ions would probably give some valuable information about the suspending fluid because these are the ions primarily contributing to the cation concentration in domestic wastewater.

The methods are modified procedures from Standard Methods for the Examination of Water and Wastewater, 1965, 12th edition.

Relative humidity

The relative humidity was determined from Psychrometer Tables (Marvin, 1941). The equipment used to determine relative humidity was:

1. Barometer—the barometric pressure was taken during the same period in which the psychrometer was used, to determine relative humidity.
2. Psychrometer—the hand aspirating psychrometer model HA-2A from the Bendix Corporation, Friez Instrument Division, Baltimore, Maryland, was used.

Temperature

The ambient air temperature, recorded in degrees centigrade, was the dry temperature measurement from the psychrometer.

Wind velocity and aerosol age

The wind velocity in conjunction with the distance from the discharge point indicated the time of exposure in which the virus was subjected to environmental stresses. The meter used to measure wind velocity was a wind measuring set, model AN/PMQ-3A from Belfort Instrument Company. The aerosol age was expressed as equivalent to the time of exposure and was determined by dividing the distance from the sprinkler source to the sampler by the wind velocity. Aerosol age is reported in seconds.

Light intensity

The light intensity was measured with a total radiation meter and reported in mcal/cm²/min. The instrument measures total radiation and was manufactured by Kahl Instruments, El Cajon, California.

Pumps and samplers

The equipment, in addition to the AGI-30 and Andersen samplers, which were used to measure the parameters important to virus decay are mentioned herein.

The pump used to regulate the Andersen sampler was a 12 volt D.C. pump from Universal Electric Co., Owosso, Michigan. Its model number was YZLUO15.

The pump used to regulate the AGI-30 was a 12 volt D.C. pump from Gast Manufacturing Corpora-

tion, Menton Harbor, Michigan. The pump model number was 0211-V208-247A.

The AGI-30 was wrapped in aluminum foil to shield the collected virus from additional exposure to light.

Fields Operations

Reason for virus addition

Viruses present in domestic wastewater can be detected when they are sprayed from a sprinkler irrigation system. Long sampling periods and/or large volumes of air would normally be required to collect the airborne virus because the concentrations of viruses in domestic wastewater are small. Inactivation of the virus during a long sampling period is a likely result of such sampling techniques and statistically accurate sampling data would be impossible to attain.

Accurate data from sampling during short periods of time can be obtained when the virus concentration in the suspending fluid (domestic wastewater) is raised. This was achieved by adding MS-2 virus to the sprinkler irrigation supply line.

The MS-2 coliphage was chosen as the virus for study to alleviate the possibility of infecting humans with airborne virus as well as other reasons discussed earlier regarding the MS-2 virus.

Point of virus addition

Several criteria were considered to determine where in the spray irrigation system the virus should be added. It was concluded that the most efficient use of the virus dictated that the virus be added to the suspending fluid a short distance from the sprinkler nozzle. The virus addition, under this condition, would minimize the physical and chemical stresses on the virus. The stresses are presented by the pipe transporting system and by the suspending fluid. The location for virus addition point must be such that the virus is in a homogeneous concentration in the suspending fluid before exiting the sprinkler nozzle.

Virus injection system

Figure 11 is a schematic diagram of a virus injection system which was developed to deliver virus to the suspending fluid at a constant rate. A description of the injection system follows.

The pipeline which transported the suspending fluid, and into which the virus was injected, delivered the wastewater suspending fluid with a high internal pressure. Because of the high internal pressure, it was

necessary to inject the virus into the pressurized system. By using pressure differentials in the pipeline, the need for electrical supply in the field was alleviated and the injection system could deliver a homogeneous concentration of the virus into the suspending pipeline fluid.

A pressure tank containing a collapsible bag of virus had the same internal pressure (40 psig) as the main pipeline. The internal pressure was transferred from the main pipeline to the pressure tank via Tygon tubing.

The collapsible bag was placed in a funnel shaped plastic support, large enough to hold the volume of virus needed for the injection period. The plastic support firmly held a rubber stopper containing a hole through which the virus could flow, and to which a collapsible bag was attached. The stopper could easily be removed from the funnel when virus was added into the collapsible bag. The funnel shaped plastic support was held in an upright position off the bottom of the pressure tank by a steel wire frame.

The virus was transferred from the pressure tank through the removable pressure tank lid via Tygon tubing. A butterfly valve was attached to the outlet side of the pressure tank and was fully opened when the virus injection began. Tygon tubing connected the valve opening to a smaller diameter pipeline. A regulatory orifice made of Teflon with a 0.059 inch hole through its middle was securely attached inside the Tygon tubing.

The 2 inch inside diameter (ID) main pipeline operated under a 40 psig internal pressure with a suspending fluid flow rate of 11.5 l/minute. When the butterfly valve was opened under these operating conditions, 300 ml/minute of virus suspension was injected into the ¼ inch I.D. pipeline. Because of the diameter reduction in the pipeline from 2 inch I.D. to ¼ inch I.D., a pressure decrease from 40 psig to 35 psig respectively was established.

When the pressure in the main pipeline was maintained, the collapsible bag was deflated and the virus concentration was injected into the reduced pressure region at a steady rate.

The pipe configuration in the reduced pressure region constricted abruptly and caused turbulent flow of the suspending fluid. Consequently the injected virus was mixed well in the suspending fluid.

A 3/4 inch I.D. pipe was attached to the ¼ inch I.D. pipe and served to hold the sprinkler nozzle which was a Rainbird model TNT-20 with a 1/8 inch diameter orifice. A suspending fluid outlet with a valve was placed in the 3/4 inch I.D. pipe and served

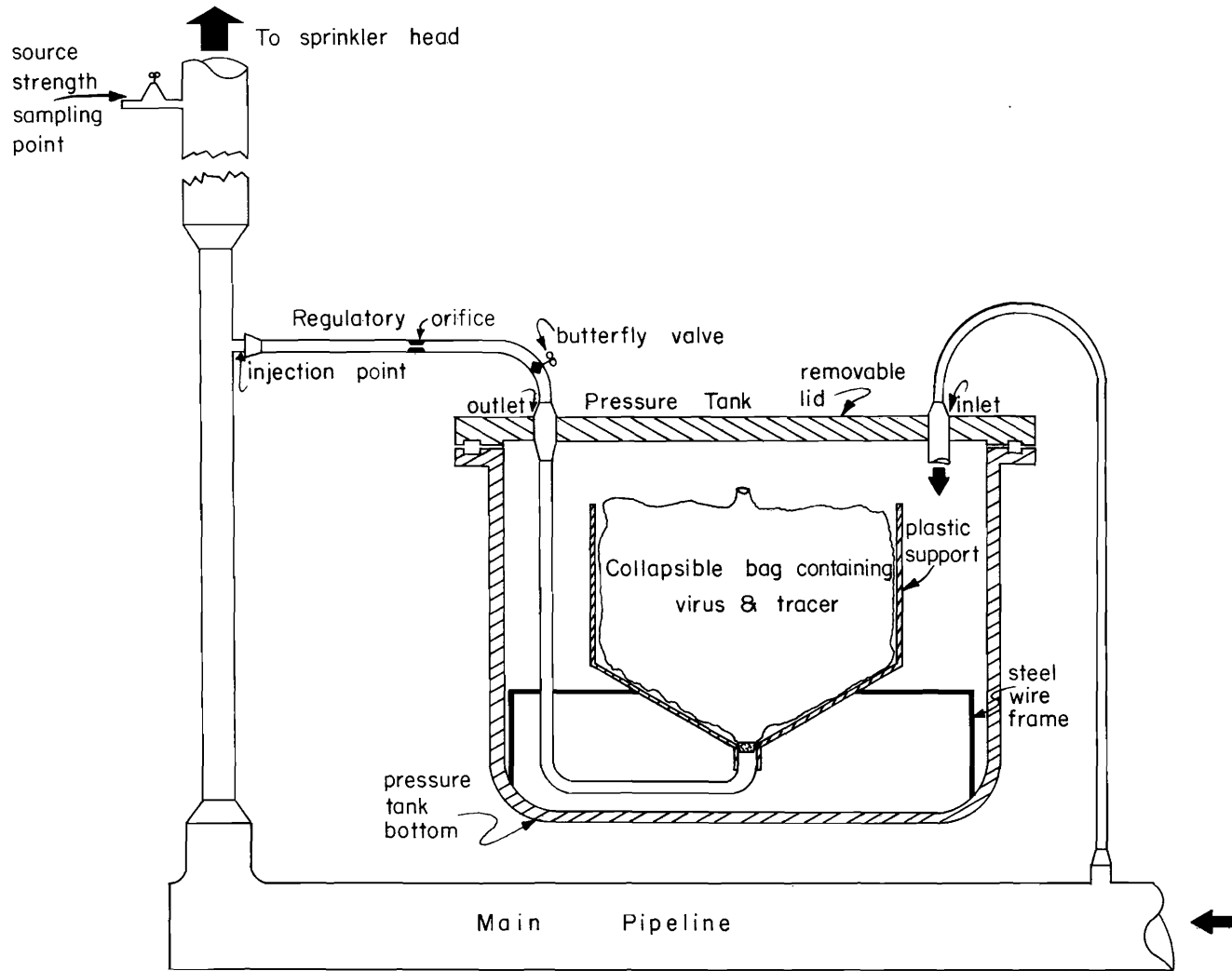


Figure 11. Schematic diagram of injection system.

as the point in the irrigation line at which a representative sample of the homogeneous concentration of virus could be taken. The liquid sample from this outlet is the sample analyzed to determine the source strength of the suspending fluid. The source strength is the concentration of virus in suspending fluid before the virus becomes airborne.

Tracer

A tracer of known concentration was mixed and injected with the virus to provide a basis for estimating the volume fraction of suspending fluid in the aerosol form which was actually collected during a sampling period. To be effective, the tracer had to have the following characteristics:

1. Concentrations had to be relatively high in the suspending fluid, in relation to analysis sensitivity.
2. It must not be affected by environmental stresses.
3. It must be collectable by the same air sampling techniques as used for the virus.
4. Assay must be easily accomplished in relatively low concentrations in relation to the assay sensitivity.
5. The virus must not be affected by the tracer's presence in solution.
6. The tracer must not be a potential threat to public health.

The resistant *B. globigii* spore's characteristics seemed to comply with the required tracer criteria. The concentration of spores can be adjusted to 1×10^9 spores/ml or more, and can be easily assayed when collected in AGI-30 collecting medium. It is not known at this time however, if the spore remains unaffected after short exposure to sunlight.

Usage alternatives for injection system

The injection system was designed to provide virus supply to a single sprinkler nozzle. The injection system, however, can be designed to supply virus to a network of sprinkler nozzles. In this case, the virus suspension would be injected in the main pipeline which supplies water to the network. Therefore, the main pipeline would contain the pipe diameter reduction. A matter of timing must be considered when planning virus injection for a network of nozzles. As the virus is injected into the main pipeline, the virus would reach the various spray nozzles at different times. Sampling should not begin until the virus laden aerosol cloud sprayed from the complex network is carried to the sample site. The volume of virus used, from the time of injection until the last spray nozzle begins spraying virus, is essentially of no value for sampling purposes. The

volume of virus actually sampled is the volume injected during the sampling period.

Timing was considered when sampling of the virus laden aerosol cloud started and ended. The time required for the virus laden aerosol to reach the samplers downwind, the exposure time, was calculated before sampling. The exposure time is found by dividing the distance from sampler station to the spray nozzle by the wind velocity. Consideration should also be given to the timing involved in ending the injection and sampling period. To insure virus homogeneity of the aerosol cloud, sampling was ceased before injection was curtailed.

Ambient air conditions

Ambient air conditions were recorded immediately before injecting the virus. The conditions measured were wind velocity, wet bulb temperature, dry bulb temperature, barometric pressure, and light intensity at time of day. Length of sample duration was also recorded.

Virus and tracer assay

Assays of the virus and tracer from the suspending fluid and AGI-30 collecting medium and virus assay of the plates from the Andersen sampler were begun immediately after the sampling period.

Contamination from microorganisms in the domestic wastewater sometimes presented a problem.

The host cell, present in high concentration, normally has a competitive advantage over extraneous organisms on the growth media. Sometimes, however, a contaminant in the air or suspending fluid may successfully compete with the host cell for the substrate. When a contaminant grew on the assay medium, it had the effect of masking the presence of virus and the sample was of no value.

The initial assay procedures consisted of preparing the AGI-30 collecting medium, Andersen, source strength and control samples for incubation. Initial assay procedures were completed in an area or shelter upwind from the spray source. When the assay plates were transferred from the field to the incubator, the plates were wrapped in waterproof plastic bags and held in an insulated container which held a large volume of water at 37° C.

Physical and chemical tests performed on the source strength were done in the laboratory and not in the field. The tests consisted of measuring the pH and solids content.

Assay Interpretation

AGI-30 and source strength— decay rate

After the virus and tracer were incubated for 12 hours and 24 hours respectively and counted, assay interpretations were made.

The AGI-30 collecting medium and source strength of the suspending fluid were analyzed for virus and tracer to measure and define the decay rate of the virus infected aerosol after the exposure time.

The concentration of infective virus in the source strength, C_{V_1}/V_{V_1} , and the number of tracer organisms in the AGI-30 collecting medium, C_{T_2} , were measured, in which

- C_{V_1} is the count of virus at source
- C_{T_2} is the count of tracer when sampled by AGI-30
- V_{V_1} is the volume in which C_{V_1} is contained in the suspending fluid

With the assumption that the tracer did not decay during airborne flight, the fraction of sprayed suspending fluid volume sampled can be calculated mathematically by

$$V_{T_2} = C_{T_2} \times \frac{V_{T_1}}{C_{T_1}}$$

in which

- C_{T_2} is the count of tracer when sampled by AGI-30
- V_{T_1} is the volume in which C_{T_1} was contained
- V_{T_2} is the volume in which C_{T_2} was collected
- C_{T_1} is the count of tracer at source

The calculation of the virus decay rate can be determined after V_{T_2} is known. The virus in the AGI-30 collecting medium contains infective viruses, C_{V_1} , and non-viable viruses, C_{V_D} . C_{V_D} represents the collected viruses which did not survive the environmental stresses during airborne flight. Therefore, the number of viruses collected by the AGI-30 consisted of C_{V_1} and C_{V_D} . When it is recognized that the fraction of suspending fluid volume of virus, V_{V_2} , in the AGI-30 collecting medium is equal to V_{T_2} , then the total amount of virus collected, C_{V_2} , can be calculated by:

$$C_{V_2} = \frac{C_{V_1}}{V_{V_1}} \times V_{T_2}$$

C_{V_D} is calculated by: $C_{V_D} = C_{V_2} - C_{V_1}$. The fraction of decayed virus, expressed as percent decay, can be calculated by:

$$\% \text{Decay} = \frac{C_{V_D}}{C_{V_2}} \times 100$$

The decay rate, R_D , is determined by:

$$R_D = \% \text{Decay} / \text{Exposure time}$$

and is reported as percent virus decay per minute.

Andersen sampler-aerosol hazard

Virus assay of the Andersen plates was performed to measure the potential hazard of virus laden aerosols from a sprinkler irrigation system. An assay of the number of plaques collected on each stage of the Andersen sampler represents the number of virus infective droplets collected from the virus laden aerosol cloud. The characteristics of the aerosol, in terms of its potential hazard to public health, is revealed by the presence of plaque forming units on the various stages of the Andersen sampler. P_{s_n} is the number of plaques found on n stage of the Andersen sampler and is reported.

The percent P_{s_n} for each stage can be calculated by

$$\%P_{s_n} = \frac{P_{s_n}}{\Sigma P_{s_n}}$$

in which ΣP_{s_n} is the sum of the P_{s_n} for six stages. The percent P_{s_n} for each stage is reported.

When the Andersen sampler results are reported in this form, it has the effect of normalizing different sample periods. A sample period of short duration may contain only a few plaques on each stage, but the sample results can be compared with a sample period of long duration when the percent P_{s_n} of each sample period is reported. If each stage of the Andersen sampler contained more P_{s_n} during the same sampling period, the interpretation would be that the decay rate of the virus was less for the higher values of P_{s_n} . The difference in decay rates for different sample periods would be detected by the AGI-30 data.

As indicated by a review of the literature, Andersen plates containing more plaques on the first two stages would represent an aerosol cloud that is more favorable to public health than a set of Andersen plates having plaques on the last four stages. In review, this is true because small particles impinged on these stages can penetrate the human lung.

CHAPTER IV

RESULTS

Methods have been developed to generate large quantities of virus laden aerosols into the ambient air and analyze the results of sampling periods.

As a verification that the virus collecting method, analytical methods, and virus injection system are useful as a coordinated system, the results of a sample run are presented from calculations of raw data and presented in Table 1.

Discussion

Sample run

Results of the analysis of the various parameters reveal that the decay rate is 33.3 percent per

minute under the various environmental stresses. However, this cannot be considered exact because the wind velocity was unsteady. The velocity was measured at less than 1 knot and was interpreted for calculation purposes as 0.5 knot.

The aerosol droplet size distribution, without benefit of comparison with other aerosols, appear to be very hazardous. This opinion is based on the fact that 84.2 percent of the virus infective particles collected by the Andersen sampler were in the hazardous range.

The decay rate was calculated by following the steps previously discussed and by using the analytical data found in Appendix A.

Table 1. Reported results—sample run.

<u>Environmental Stresses</u>	
1.	<u>Suspending Fluid</u>
	Dissolved Inorganic Salt Content 31.8 mg/l
	% Dissolved Inorganic Salt 29.4%
	Dissolved Organic Content 76.2 mg/l
	% Dissolved Organic Content 70.6%
	Filterable Solids 30.1 mg/l
	pH 5.4
2.	<u>Ambient Air Conditions</u>
	Relative Humidity 33%
	Temperature 17°C
	Solar Radiation 0 mcal/cm ² /min
	Aerosol Age 58 seconds
	Decay Rate 33.3% virus decay/minute
<u>Aerosol Hazard</u>	
Stage n	P _{sn}
1	2
2	7
3	17
4	2
5	17
6	12
Stage n	%P _{sn}
1	3.5
2	12.3
3	29.3
4	3.5
5	29.8
6	21.1

$$V_{T_2} = C_{T_2} \times \frac{V_{T_1}}{C_{T_1}}$$

$$= 20 \times \frac{1}{2 \times 10^7}$$

$$= 1 \times 10^{-6} \text{ ml}$$

$$C_{V_2} = \frac{C_{V_1}}{V_{V_1}} \times V_{T_2}$$

$$= \frac{1.5 \times 10^7}{1} \times 1 \times 10^{-6}$$

$$= 15 \text{ PFU}$$

$$C_{V_D} = C_{V_2} - C_{V_1}$$

$$= 15 - 10$$

$$= 5 \text{ PFU}$$

$$\% \text{Decay} = \frac{C_{V_D}}{C_{V_2}} \times 100$$

$$= \frac{5}{15} \times 100$$

$$= 33.3\%$$

$$\text{Exposure Time} = \frac{\text{distance}}{\text{wind velocity}}$$

$$= 56.8 \text{ sec. (see Appendix A for calculations)}$$

$$\text{Decay Rate, } R_D = \frac{\% \text{ decay}}{\text{exposure time}}$$

$$= \frac{33.3\%}{1 \text{ min}}$$

$$= 33.3\% \text{ per minute}$$

Source strength homogeneity

Table 2 contains a set of data obtained from the source strength sampling point during an injection period. The test was designed to measure the extent to which the injection system is able to produce a homogeneous suspending fluid. A high salt concentration was injected from the collapsible bag in the pressure tank into the injection point in the smaller diameter pipe. The operating conditions during which the samples were collected were the same as those at which the virus would normally be injected; e.g., 40 psig in the main pipeline and 35 psig at the injection point. Electroconductivity of the source strength was measured. The control sample which had a lower conductance value than the other samples, was taken

before injection began. A comparison between the control and the other samples indicates that the salt was indeed injected. All samples were collected in 100 ml beakers which contained about 75 ml of sample and collected over about a 30 second period. Sample number one was taken about one minute after injection began and all subsequent samples were on a continuous basis.

Table 2. Injection samples tested for homogeneity.

Sample Number	Conductance (Micromhos)
Control	261
1	1230
2	1230
3	1230
4	1210
5	1230
6	1210
7	1250
8	1230
9	1240
10	1230

A statistical analysis of the data from Table 2 reveals that the injection system produced a homogeneously mixed suspending fluid at the source strength outlet.

The arithmetic mean, \bar{x} , of the data points was 1229.00 and was found by the formula

$$\bar{x} = \frac{\sum_{j=1}^N N_j}{N}$$

in which

- j = individually numbered data points
- N = total number of data points = 10
- x_j = specific sample number, j

The standard deviation, s , of these data was calculated to be 11.97 by the formula:

$$s = \sqrt{\frac{\sum_{j=1}^N (x_j - \bar{x})^2}{N-1}}$$

The coefficient of variation, CV, of these data was calculated to be 0.97 percent by the formula:

$$CV = \frac{s}{\bar{x}} (100)$$

CHAPTER V

DISCUSSION

Application of Data

Decay rate

The results of the test run indicate that the decay rate was 33.3 percent virus decay per minute. The decay rate was calculated from a sample run that was meant as a demonstration run and has no statistical accuracy.

If it is assumed that the analysis of the suspending fluid represents a typical domestic wastewater solids analysis, some conclusions related to the decay rate can be made.

In a region where a summer day may be 17° C, decay rate of the virus laden domestic wastewater aerosol can be used to define a buffer zone around the sprinkler irrigation site. If it is assumed that sprinkler irrigation of domestic wastewater is done in sunlight, the buffer zone will include a safety factor because the sunlight effect was minimal in the decay rate calculations.

A buffer zone can be defined after extensive studies of virus decay have been completed under varying environmental conditions. The definition of a buffer zone should be a function of the probability of a person, exposed to the infective aerosol, inhaling a single virus. The probability of inhalation in an area is a function of the sprinkler irrigation plot size and one would expect the probability of inhalation to increase with an increased density of infective droplets in a volume of air.

For purposes of defining a buffer zone, an arbitrary decision was made to define the zone as the distance from the outside sprinklers of the irrigation plot through which the prevailing daytime winds would carry an infective aerosol to 99 percent decay and in which public access would be prohibited.

It is assumed that the effect of environmental stresses on the virus causes an exponential decay rate. Therefore the distance required for the definition of a buffer zone is found by the following steps:

$$\% \text{ Decay} = e^{K/t}$$

in which

- K is a constant for the prevailing environmental conditions of ambient air and domestic wastewater
- t is exposure time equal to distance from source during exposure time divided by wind velocity, or $t = d/v$ and is in seconds.

Since the decay rate was 33.3 percent per minute, K can be found by:

$$\begin{aligned} \ln (\% \text{ Decay}) &= K/t \\ \ln (0.33) &= K/60 \text{ seconds} \\ K &= -66.5 \end{aligned}$$

When K is known, the distance required for a buffer zone around a sprinkler irrigation field is found by:

$$\begin{aligned} \ln (0.99) &= K \frac{v}{d} \\ d &= \frac{(K)(v)}{\ln (0.99)} \end{aligned}$$

for 99 percent decay, $d = 5,586$ feet when wind velocity is 0.844 feet per second (0.5 knots).

Buffer zone

Figure 12 represents the percent of MS-2 virus that would have decayed over a distance from the source, assuming a K value of -66.5 and a constant velocity of 0.844 ft/sec. The data points were taken from Table 3, columns 1 and 3. It can be seen from Figure 12 that the buffer zone required would be 5,584 ft.

Figure 13 represents the percent of infective MS-2 virus that would be expected to survive over a distance, assuming a K value of -66.5 and a constant velocity of 0.844 ft/sec. The data points were taken from Table 3, columns 2 and 3. The figure serves to

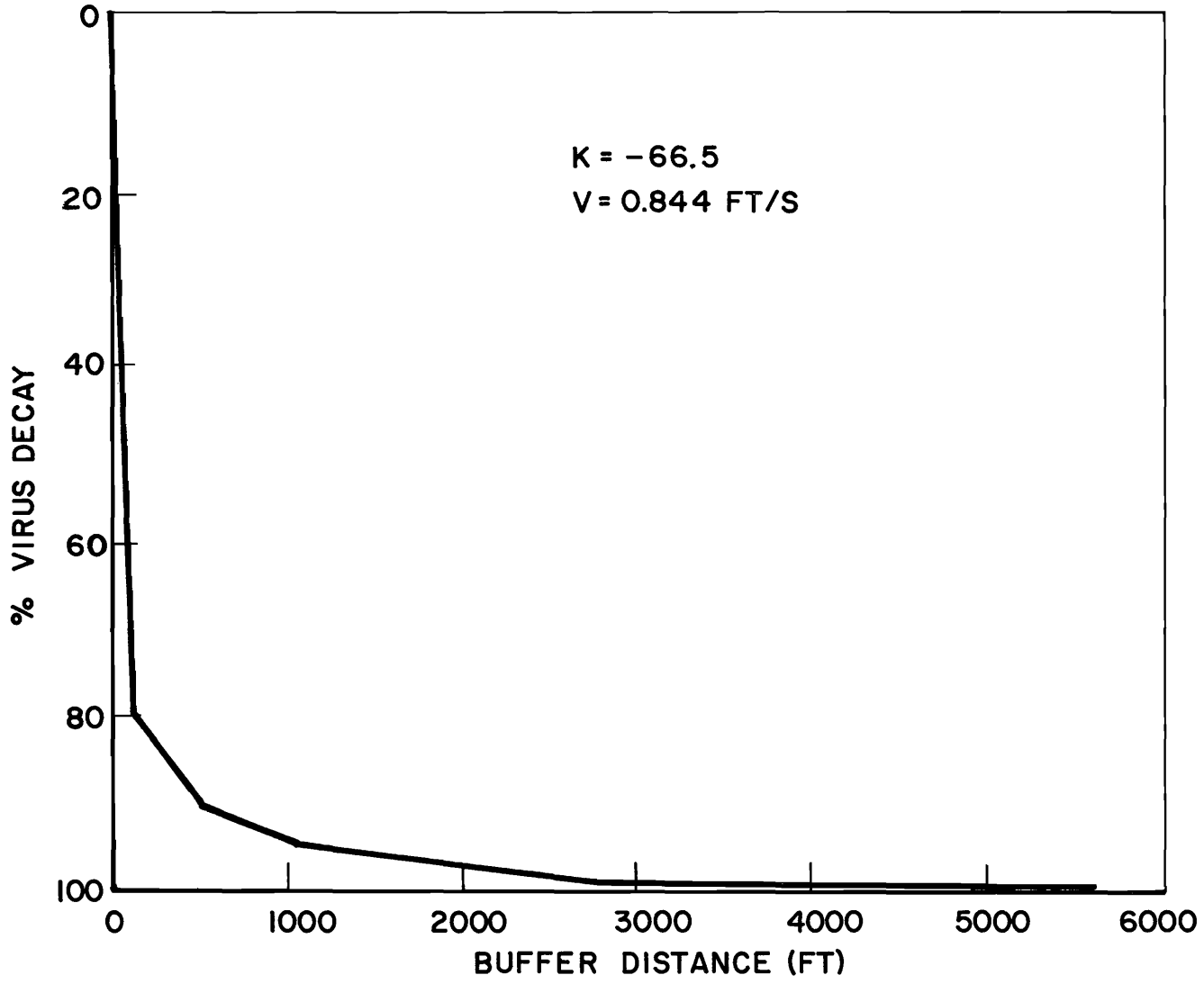


Figure 12. Percent decayed virus vs buffer distance.

Table 3. Buffer distance calculations.

Column	1	2	3	4	5
	% Decay	% Remaining	Distance (ft)	MS-2 Remaining	PFU Remaining (from Literature)
	0	100	0	146 x 10 ⁵ /ml	11
	10	90	24	131.4 x 10 ⁵ /ml	
	20	80	35	116.8 x 10 ⁵ /ml	8.8
	30	70	47		
	40	60	61	87.6 x 10 ⁵ /ml	6.6
	50	50	81		
	60	40	110	58.4 x 10 ⁵ /ml	4.4
	70	30	157		
	80	20	252	29.2 x 10 ⁵ /ml	2.2
	90	10	533		
	95	5	1094	7.3 x 10 ⁵ /ml	
	99	1	5584	1.46 x 10 ⁵ /ml	0.11

complement Figure 12, to show 1 percent survival at a 5,584 ft buffer distance.

Figure 14 represents the concentration of infective MS-2 virus/ml remaining at various distances, assuming a K value of -66.5 and a constant velocity of 0.844 ft/sec. The data points were taken from Table 3, columns 3 and 4. It can be seen that 1.46 x 10⁵/ml of MS-2 would survive through the buffer distance. The AGI-30 collected 20 PFU in 5 minutes at about 50 feet from the source and sampled 2.2 cubic feet of air. If a person inhaled all the MS-2 breathed, and 20 cubic feet of air per minute was breathed, theoretically 909 infective MS-2 particles would have been inhaled. This represents approximately 0.0069 percent (909/131.4 X 10⁵) of virus that theoretically had survived to this distance from the source. If this dispersion were maintained until 99 percent decay, a person theoretically would inhale 10 PFU in the infective aerosol cloud at the threshold of the buffer zone during the sampling period.

Figure 15 represents the concentration of viruses/ml over a buffer distance. The concentration is based on literature reports from Shuval (1970). The figure assumes that the viruses in domestic wastewater undergo the same rate of decay as MS-2. The data points were taken from Table 3, columns 3 and 5. Following the same discussion as in Figure 14, a person would be expected to inhale (0.0069% X 1.1) 7.59 X 10⁻⁵ PFU at the 5,584 feet buffer distance. From this value, it can be calculated that a person can breathe the aerosol cloud for 5/7.59 X 10⁻⁵ (60) = 1,097 hours before 1 PFU is inhaled. The time value, of course, was calculated for an aerosol cloud produced from one sprinkler. A large sprinkler irrigation system would produce a more concentrated cloud and a person could not breathe the aerosol as long as calculated before 1 PFU would be inhaled.

The calculated exposure time, however, does indicate that 99 percent decay may not be required to define a buffer zone. A more reasonable distance, from observing Figure 15, would be 3,000 feet where the virus would have theoretically undergone approximately 96 percent decay. Obviously, the area required for a buffer zone under 96 percent decay is significantly less than that required under 99 percent decay.

Figure 16 represents the percent of viruses remaining over a distance assuming a K value of -66.5 and varying wind velocities from 5 miles per hour (7.33 ft/sec) to 40 miles per hour (58.67 ft/sec). The data points for Figure 16 were taken from calculations in Table 4. It can be observed that increased prevailing wind velocities make a significant difference in defining the buffer zone. From this observation, it can be concluded that economics would require a domestic wastewater sprinkler irrigation system to be located and operated where and when the prevailing wind conditions have a low velocity.

K value discussion

The K value does not account for sunlight affect nor the fact that the aerosol age affects the virus infected aerosol in an exponential manner.

Using a K value of -66.5 and a wind velocity of 0.844 ft/sec, a buffer zone around a sprinkler irrigation plot has been defined as 5,586 feet using 99 percent virus decay in the aerosol.

Table 5 represents the percent of land used as a buffer zone with various sprinkler irrigation plot sizes. Figure 17 represents the calculations from Table 5.

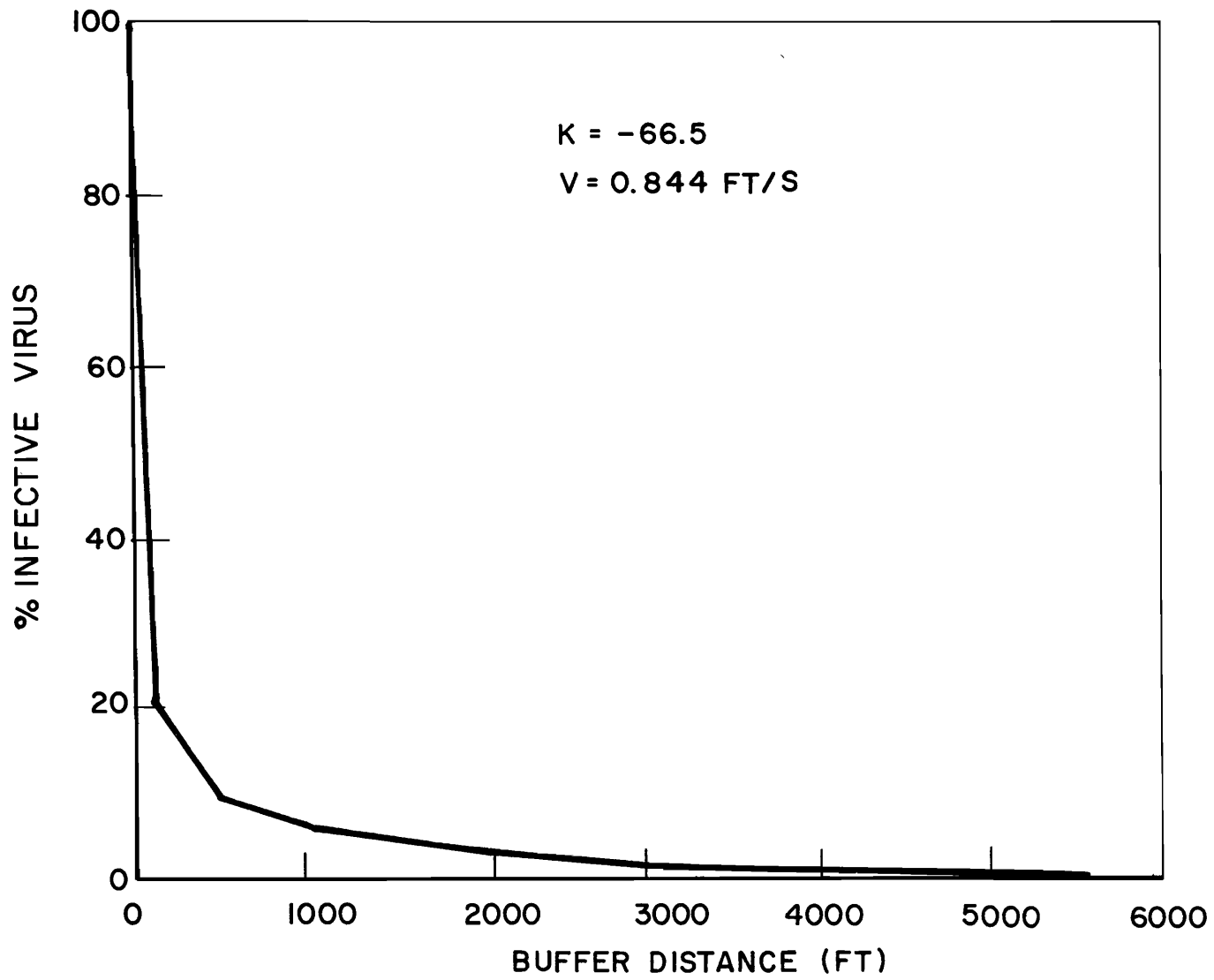


Figure 13. Percent infective virus/ml versus distance from sprinkler source.

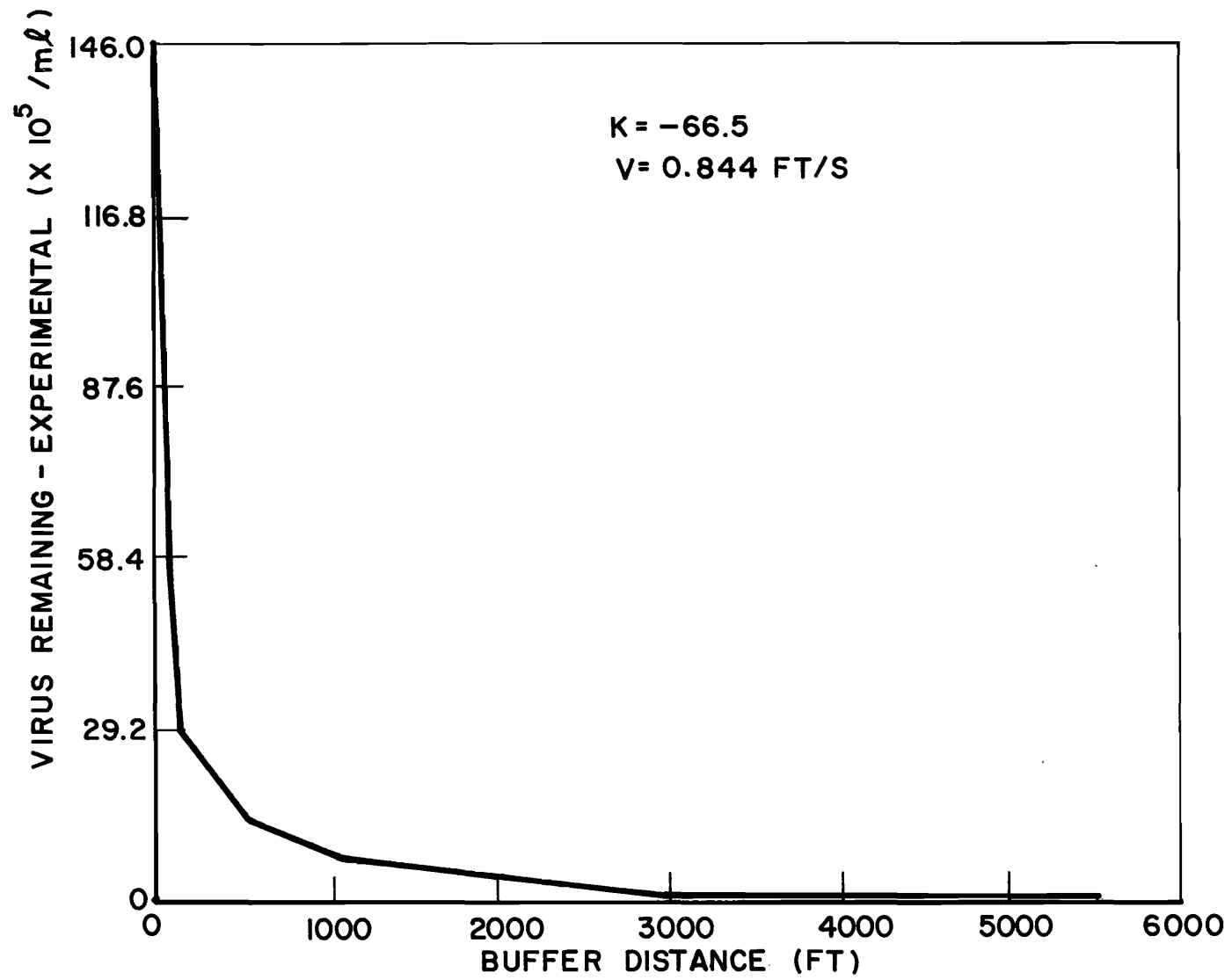


Figure 14. Experimental number of viruses remaining/ml versus buffer distance.

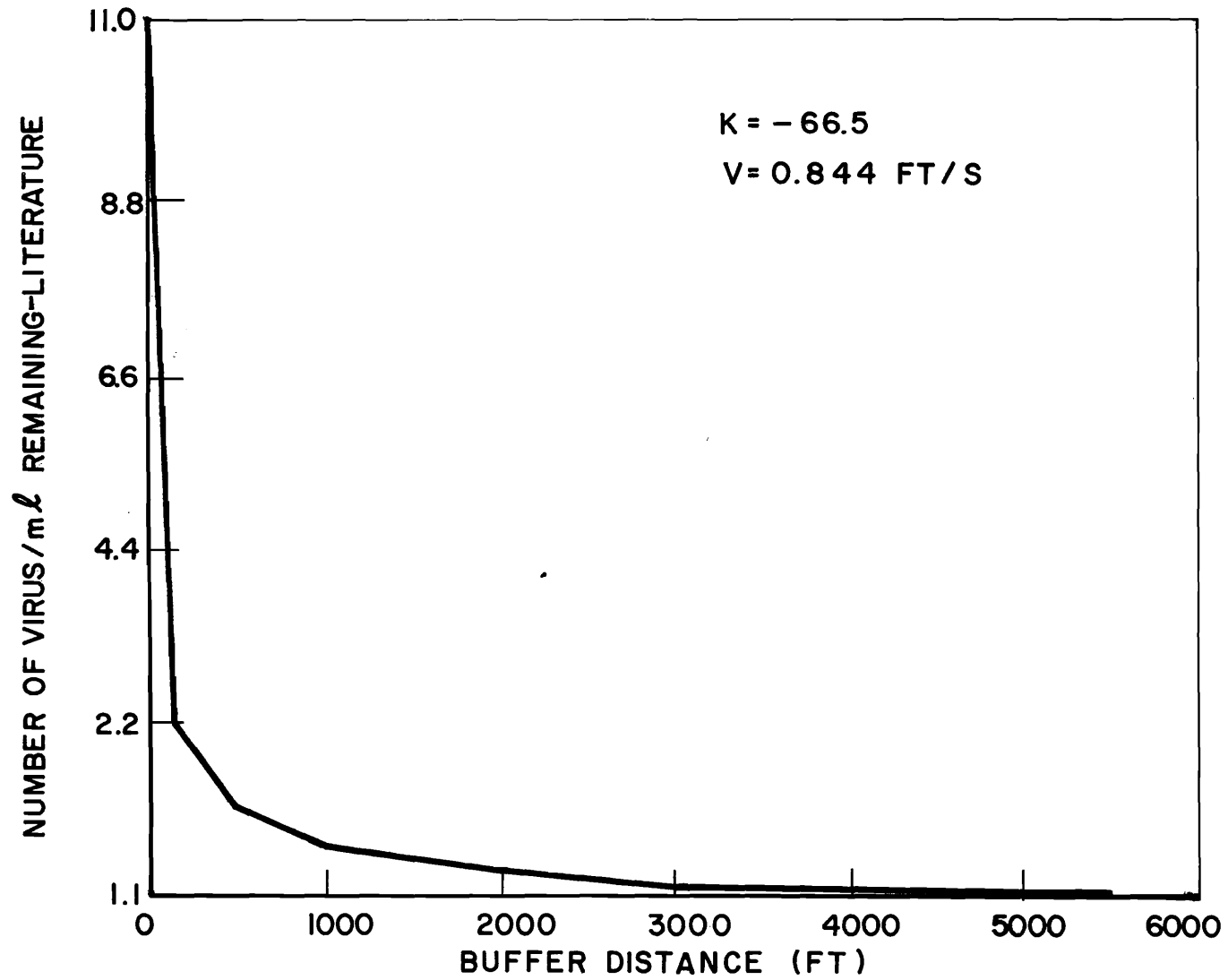


Figure 15. Number of viruses remaining (from literature criteria) versus distance.

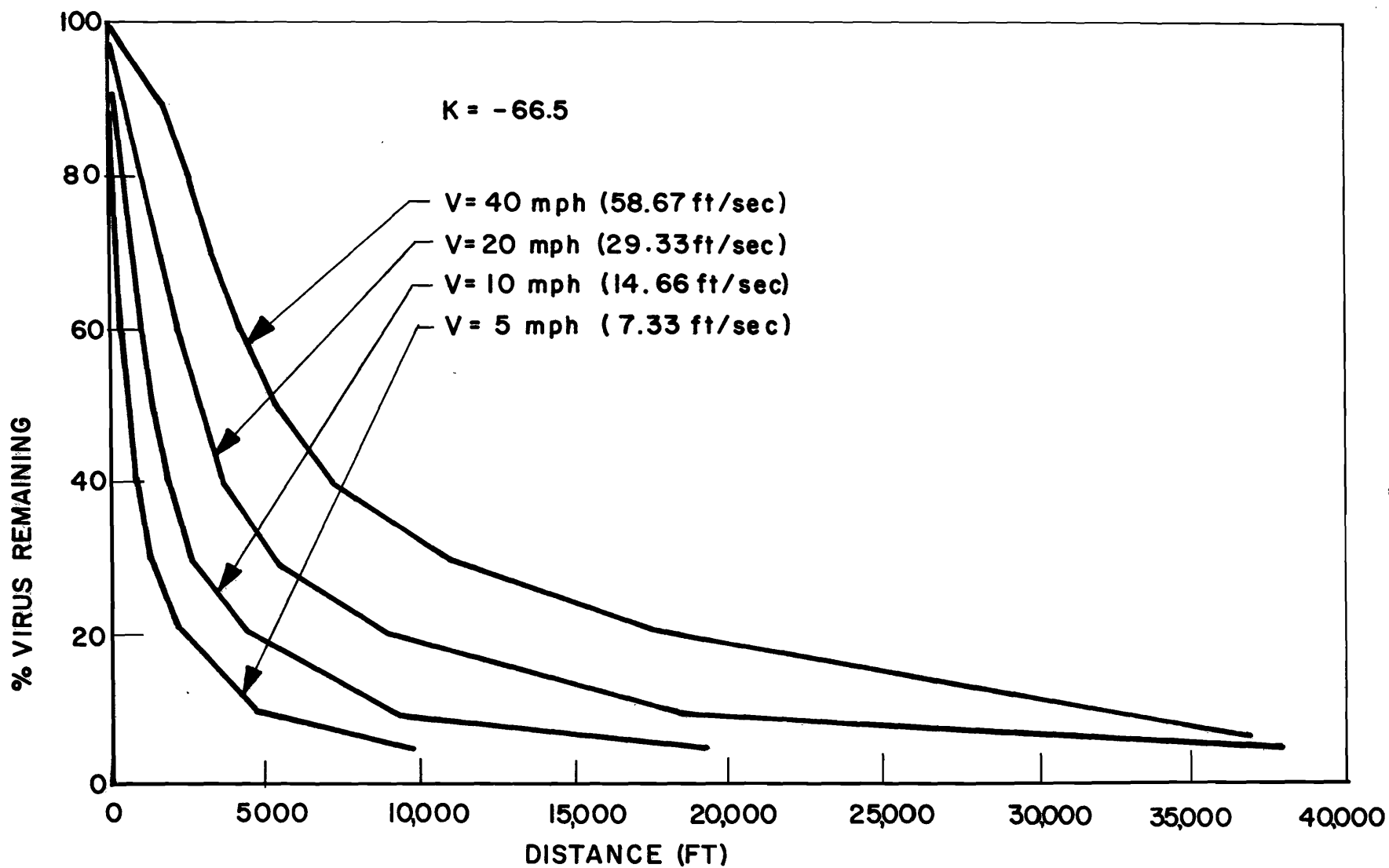


Figure 16. Percent infective virus versus distance with various wind velocities.

Table 4. Buffer zone with varying wind velocities.

% Decay	% Remaining	Distance (ft/s)			
		(7.33)	(14.66)	(29.33)	(58.67)
10	90	212	424	848	1,696
20	80	303	606	1,212	2,424
30	70	404	808	1,616	3,232
40	60	532	1,064	2,128	4,256
50	50	703	1,406	2,812	5,624
60	40	954	1,908	3,816	7,632
70	30	1,367	2,734	5,468	10,936
80	20	2,184	4,368	8,736	17,472
90	10	4,626	9,252	18,505	37,008
95	5	9,503	19,006	38,012	76,024

Table 5. Plot size vs. percent buffer area.

Sprinkler Irrigation Plot (sq. miles)	Buffer Area (sq. miles)	% Buffer Area
1	8.71	89.7
4	12.94	76.4
9	17.17	65.6
16	21.40	57.3
25	25.64	50.6

Table 6. Characteristic droplet diameter and volume on Andersen sampler stages.

Stage	d (μm)	V _n (μm ³)
1	12.15	939.1
2	7.75	243.7
3	4.4	44.6
4	3.0	14.1
5	1.5	1.8
6	0.9	0.4

The economics of a sprinkler irrigation system plot size can be viewed in terms of the fraction of the total plot which is required for buffer zone area. As the area for the sprinkler irrigation field becomes larger, the fraction of area required for the buffer zone becomes smaller. The calculations were made on the assumption that the sprinkler irrigation plot configuration is square, the most efficient four sided configuration.

Theoretical Considerations

AGI-30 and Andersen sampler

The total volume of droplets collected on each Andersen sampler stage, ΣV_{s_n} , can be calculated by multiplying the characteristic volume of the droplet found on each Andersen stage by the number of plaque forming units on the particular stage. The characteristic volume of each stage's droplet can be calculated by $V_n = (\pi d^3)/6$ if it is assumed that the droplets are spherical in shape. The characteristic droplet diameter of each stage, d_n , has been discussed previously and is reviewed in Table 6 with the characteristic droplet volume, V_n , which was col-

lected on each stage. A discussion of the Andersen sampler's use as an aerosol hazard index will follow.

The volume of PFU per stage, V_{s_n} , can be calculated by

$$V_{s_n} = P_{s_n} \times V_n$$

When the sum of the V_{s_n} for the six stages $1/\%V_{s_n}$ of the Andersen sampler are calculated, ΣV_{s_n} , the volume percentages of each stage, $\%V_{s_n}$, can be calculated by

$$\%V_{s_n} = V_{s_n} / \Sigma V_{s_n} \times 100$$

Because the volume of a particle is inversely proportional to the droplet's infectivity potential, the inverse of $\%V_{s_n}$ is also reported. This value can be reported and used as an index of the relative hazard of a virus infective aerosol.

When potential aerosol hazards of aerosol clouds from different sampling periods are evaluated, comparisons of the V_{s_n} from each stage and of the entire set of the six V_{s_n} stages can be made.

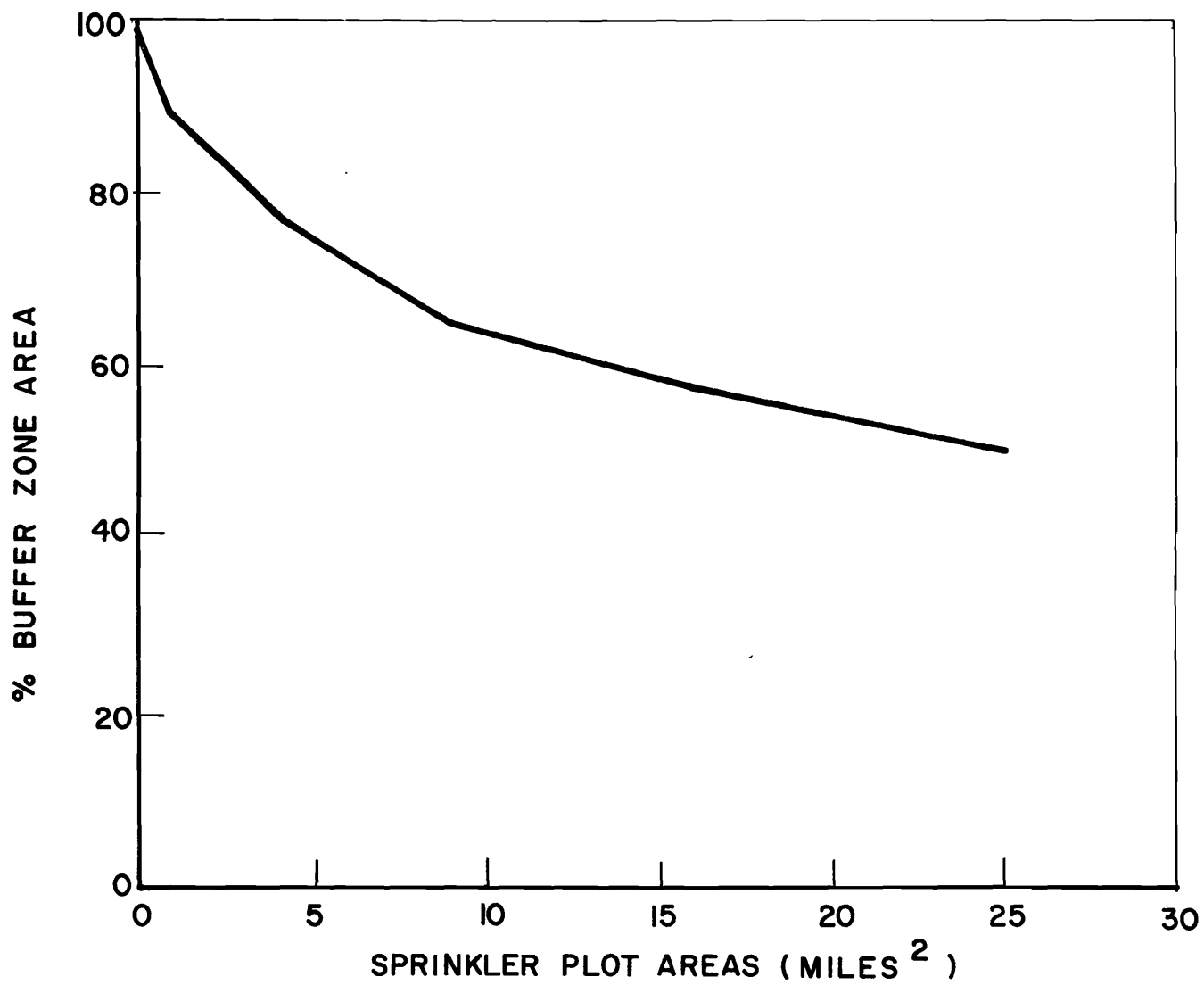


Figure 17. Percent buffer zone area versus sprinkler plot areas.

Theoretical method to characterize virus laden aerosols

The Andersen sampler and AGI-30 were located next to each other during the sampling period and sampled essentially the same aerosol cloud. From this concession, some inferential calculations can be made about the potential aerosol hazard by comparing the analytical data from the Andersen sampler and AGI-30. The calculations are made to describe the aerosol in terms of viruses per droplet. This can be done after the virus concentration of the AGI-30 is normalized mathematically. The Andersen sampler collected 1 cfm during the period that the AGI-30 collected 0.44 cfm.

If it is assumed that the AGI-30 operated at 50 percent efficiency relative to the Andersen sampler, a $C_{V_1}(\text{New})$ can be calculated by:

$$C_{V_1}(\text{New}) = C_{V_2} / (0.44 \times 0.5)$$

$C_{V_2}(\text{New})$ represents the quantity of viruses that would have been collected, had the AGI-30 operated at 1 cfm with the same efficiency as the Andersen sampler.

A relative estimate of the number of infective viruses can be calculated if it is assumed that all droplet sizes contain equal amounts of virus per volume. The aerosol, when generated from the source, by the nature of the injection system, contained equal amounts of virus per volume. However, during evaporation, an arithmetic decrease in the diameter of the droplet is the same as decreasing the droplet volume geometrically, and the result is a geometric increase in viruses per droplet volume. Therefore, the above assumption is not exactly valid. With this known, an index may be established, using the assumption, which may serve to indicate the relative number of viruses that could be inhaled during the sampling period.

The relative number of infective viruses per volume, I_v , on the Andersen sampler stages can be calculated by:

$$I_v = \frac{C_{V_1}(\text{New})}{\sum V_{s_n}}$$

in which V_{s_n} is the sum of the PFU volumes counted on each stage of the Andersen sampler.

The theoretical number of infective viruses per stage, I_{s_n} , of the Andersen sampler can be calculated by:

$$I_{s_n} = I_v \times V_{s_n}$$

When the sum of the I_{s_n} for the six stages of the Andersen sampler are calculated, $\sum I_{s_n}$, the virus percentages of each stage, $\%I_{s_n}$, can be calculated by:

$$\%I_{s_n} = \frac{I_{s_n}}{\sum I_{s_n}} \times 100$$

The $\%I_{s_n}$ is exactly equal to $\%V_{s_n}$ because the droplets are assumed to contain homogeneous concentrations of viruses. The relative value of $\%I_{s_n}$ is inversely proportional to the infectivity potential and the inverse of $\%I_{s_n}$ can be used as an index of the relative hazard of a virus infected aerosol. Because homogeneity of virus concentration is assumed $1/\%I_{s_n}$ is equal to $1/\%V_{s_n}$.

It should be remembered that the value $\%I_{s_n}$ and $\%V_{s_n}$ are relative numbers because the number of viruses per droplet cannot be calculated by using the analytical methods which were discussed in this report. If techniques are developed to calculate the number of viruses per particle, this writer suggests the use of $\%V_{s_n}$ and $\%I_{s_n}$ will become more valuable than using $\%P_{s_n}$ as a tool for describing the virus infective aerosol characteristics.

CHAPTER VI

SUMMARY

A technique was described which, when used, would optimize the reliability of data collected under field conditions when measuring virus laden aerosols generated from domestic wastewater sprinkler irrigation. The accuracy of the data was shown to be able to be optimized by injecting high concentrations of a human virus simulant, MS-2 coliphage into sprinkler irrigation fluid. A description of the technique included a means to measure the environmental factors which are known to influence virus decay in aerosols and a method to collect virus laden aerosols with an all glass impinger (AGI-30) and Andersen sampler. The use of the samplers were shown to provide data which could be used to calculate virus laden aerosol decay rates and describe the potential hazard of an aerosol cloud respectively.

By demonstrating the technique, it was calculated that the virus laden aerosol underwent 33.3

percent decay per minute at 33 percent relative humidity, 70.6 percent dissolved organic material, 29.4 percent inorganic, 30.1 mg/l filterable solids, 17°C ambient air temperature with no solar radiation. The aerosol cloud appeared to be a potential health hazard because 84.2 percent of the virus laden droplets collected by the Andersen sampler were in the size range that could be inhaled.

The use of the technique was shown to be valuable to the environmental engineer. When the decay rate of the virus under prevailing environmental conditions is known, it was shown that a buffer zone could be defined. The buffer zone was defined, for discussion purposes, as the area around the sprinkler irrigation site in which the virus laden aerosol was expected to undergo a certain amount of virus decay and in which public access is prohibited.

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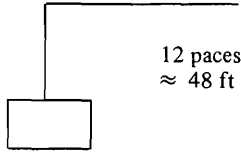
Appendix A Raw Data Sample Run

DATE 17 May 1975
 SAMPLE NUMBER 1
 DURATION OF SAMPLE 5 min. TIME 9:35 P.M.
 BAROMETRIC PRESSURE 30-29
 RELATIVE HUMIDITY DRY 63 °F 17 °C
 WET 49 °F
 LIGHT INTENSITY 0 mcal/cm²/min
 WIND VELOCITY < 1 Knot

SOURCE STRENGTH	C _{V₁} total virus	C _{T₁} total tracer
10 ⁻⁵	146x10 ⁵ = 1.5x10 ⁷	TNTC
10 ⁻⁷	0	2 x 10 ⁷
10 ⁻⁹	0	0
10 ⁻¹¹	0	0
10 ⁻¹³	0	0

COMMENTS

wind unsteady



12 paces
≈ 48 ft

Exposure time:
 assume V = 0.5 Knots = .844 ft/sec
 $t = d/v$
 48 ft x 1/(0.5 (6080 ft/hr)) x 3600 sec/hr
 = 56.8
 ≈ 60 sec.

SOURCE SOLIDS

Aliquot (ml)	SOLIDS	FINAL	INITIAL	DIFF	mg/l
30	Filtered	7.6311	7.6220	9.1	30.1
33	TDS organ + inorgan	28.4368	28.4008	36.0	108
33	Combust. Organ.	28.4368	28.4114	25.4	76.2
	Fixed Inorganic				31.8

CONTROLS

Source Tracer
 Source Virus
 Ambient Air
 Positive Virus
 Positive Tracer
 Bottom Agar Virus
 Top Agar Virus
 Host Virus
 Impinger Virus
 Agar Tracer
 Dil. Water Virus
 Dil. Water Tracer

COLLECTION

	C _{V_I} virus	C _{T_I} tracer
10 ⁻¹	1	2
10 ⁻²	0	0
10 ⁻³	0	0
10 ⁻⁴	0	0
10 ⁻⁵	0	0

Source pH 5.4

ANDERSEN SAMPLER (PFU'S/STAGE)

STAGE n	Count	ADJUSTED COUNT - P _{s_n}
1	2	2
2	7	7
3	17	17
4	2	2
5	17	17
6	12	12

Appendix B

Definition of Symbols

C_{T1}	count of tracer at source	V_{sn}	the volume of PFU per stage, n, of the Andersen sampler
C_{T2}	count of tracer in AGI-30 sampling medium	V_{T1}	the volume in which C_{T1} was contained
C_{V1}	count of infective viruses in AGI-30 sampling medium	V_{T2}	the volume in which C_{T2} was contained
$C_{V1} \text{ (New)}$	theoretical count of infective viruses that would have been collected by the AGI-30 if the sampler operated at 1 cfm and 50 percent efficiency compared to the Andersen sampler	V_{V1}	the volume of virus in which C_{V1} was contained
C_{VD}	theoretical count of non-infective viruses in the AGI-30 sampling medium	V_{V2}	the volume of virus in which C_{V2} was contained
C_{V1}	count of virus at source	$\%I_{sn}$	the fraction, expressed as percent, of the theoretical number of viruses found on each stage, n, of the Andersen sampler
C_{V2}	count of infective and non-infective viruses in the AGI-30 sampling medium	$\%P_{sn}$	the fraction, expressed as percent, of the total number of PFU found on n stage of the Andersen sampler
d_n	mass median diameter of droplet on stage n of Andersen sampler	$\%V_{sn}$	the fraction, expressed as percent, of the total volume of infective droplets collected on stage n of the Andersen sampler
I_{sn}	theoretical number of infective viruses per Andersen stage	ΣI_{sn}	summation of the theoretical number of viruses found on each stage of the Andersen sampler
I_V	relative number of infective viruses per volume	ΣP_{sn}	the summation of the number of PFU found on each stage, n, of the Andersen sampler
P_{sn}	the number of plaques found on n stage of the Andersen sampler	ΣV_{sn}	the summation of the total volume of droplets collected on each stage, n, of the Andersen sampler
R_D	decay rate of the virus infective aerosol cloud		
V_n	volume of the droplet collected by n stage of the Andersen sampler		

