Effect of Sampling Time on the Total Recovery Rate of AGI - 30 Impingers for *E. coli* Aerosols

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This study attempted to ascertain how physical factors, including inlet shape, sampling flow rate, sampling time, and number of impingers connected in series, affected the total recovery rate of AGI-30 impingers for *Escherichia coil* (*E. coli*) aerosols. A nebulizer was employed to generate *E. coli* aerosols to measure the total recovery, which is defined as the ratio of the colony forming units (CFU) in a collected sample to the concentration of bacterial particles in the air from which the sample was extracted. During each test, the relative humidity and temperature in the test chamber were maintained at 55 ± 5% and 22 ± 2℃, respectively. Samples were diluted in a series of 10 folds, seeded on agar plates, and then incubated at 37℃ for 24 hours for subsequent colony counting. Single impingers with both a curved and a straight inlet had a similar total recovery. For sampling flow rates in the range of 6-13 L/min, the maximum total recovery of single impingers with either inlet type was at 13 L/min. Measurements of various sampling times revealed that the total recovery of single impingers with either inlet had a maximum at 2.5 minutes. The combined total recovery of two impingers connected in series increased with flow rate, and the total recovery of the second impinger ranged from 47-73% of the total recovery of the first one.

Keywords: impinger, total recovery, sampling time, *E. coli*, bioaerosol

1. Introduction

As the warm and humid subtropical climate of Taiwan is particularly suited to the production of microorganisms, the characterization of health-related viable bioaerosols in residential and occupational environments is of significant interest. Bioaerosol characterization requires that a representative sample in which the microorganisms remain viable be obtained. Owing to the stress imparted to microorganisms during sampling, more sensitive bacterial strains may not survive. The overall efficiency of bioaerosol sampling therefore depends on the hardiness of microorganisms as well as the physical collection efficiency. In a comprehensive review on the evaluation of samplers for microbiological aerosols, Henningson and Ahlberg (1994) concluded that there was a need for standardized methods and recommended procedures.

To avoid uncertainties that arise from uncontrolled or unknown bioaerosol concentrations and particle size within natural environments, the performance of bioaerosol samplers should be assessed in a laboratory through test aerosols.

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Herein, AGI-30 impingers (Ace Glass, Inc., Vineland, NJ, USA) were employed to evaluate the total recovery rate for *E. coli* aerosols, which are a sensitive bacterial strain that were prepared in our laboratory. The AGI-30 impinger was developed to sample bioaerosols (May and Harper, 1957) and is considered a reference bioaerosol sampler (Brachman *et al.* 1964). The jet nozzle in the impinger is positioned above the collection liquid to provide an impact surface that is softer than the glass bottom of the impinger. An impinger sample can be diluted at various ratios for subsequent incubation and colony counting. Numerous parameters including sampling flowrate and sampling time affect the sampling efficiency of an AGI-30 impinger (see, for example, Jensen *et al.*, 1992; Juozaitis *et al.* 1994). Therefore, the primary objective of this study was to determine the effects of inlet shape, sampling flowrate, sampling time and the number of impingers connected in series on the total recovery rate of *E. coli* aerosols.

2. Materials and Methods

To generate the test aerosols, an active *E. coli* culture was inoculated into trypticase soy agar (TSA, Difco, Detroit, MI, USA) and incubated at 37℃ for 24 hours (see, for example, Jensen *et al.*, 1992). The colonies that formed were transferred aseptically to a centrifuge tube, diluted with 5-ml sterile deionized water, and centrifuged at 2,500 rpm for 5 minutes. The supernatant was discarded and the pellets resuspended in sterile deionized water. Centrifugation and resuspension were performed twice. A three-jet Collison nebulizer (BGI, Inc., Waltham, MA, USA) with an air flowrate of 3 L/min was employed to generate *E. coli* aerosols from a liquid suspension of the bacteria. The bioaerosol that was generated was then applied in sampling experiments that included single impingers as well as two impingers connected in a series. Notably, the glass impingers had a jet-to- plate distance of 30 mm. Two types of impinger inlets were examined: bent and straight. The sampling flow rate ranged from 6 to 13 L/min (the velocity at the nozzle ranged from 128 to 277 m/s), while sampling time ranged from 1 to 5 minutes.

The experimental system, which was based on that of Li *et al.* (1999), consisted mainly of an aerosol generator, a diffusion dryer, a Kr-85 particle charge neutralizer (TSI, Inc., St, Paul, MN, USA), and a test chamber. The aerosol stream at 3 L/min emitted from the charge neutralizer was diluted with a filtered air stream at 50 L/min. The diffusion dryer facilitated the water evaporation from droplets and therefore the resulting aerosol would consist of dry bacterial particles. The charge neutralizer brought the bacterial particle charges to the Boltzmann equilibrium so that the charge effect on sampling would be minimized. The system was placed in a ventilated cabinet. The test chamber was a column, which was 12.5 cm diameter by 27 cm height. During the experiment, the relative humidity and temperature in the test chamber was maintained at 55 ± 5% and 22 ± 2 ℃, respectively.

The collection liquid consisted of 1% peptone (Difco, Detroit, MI, USA), 0.01% Tween 80 (a wetting agent) and 0.005% antifoam A (Sigma Chemical Co., St. Louis, MO, USA) in deionized water. The wetting agent facilitated bacteria dispersion and the antifoam agent reduced water evaporation. Following sampling, the liquid was removed and the impinger rinsed thoroughly with fresh collection liquid. The impinger and rinsing liquids (20 ml in total volume) were mixed and stirred thoroughly. A series of 10-fold dilution was prepared with 1,000 μL of the resultant suspension. The dilution liquid consisted of 0.1% peptone in deionized water. Subsequently, 200 μL of both the diluted and undiluted suspensions

were plated on trypticase soy agar and incubated at 37℃ for 24 hours.

The number of colonies formed on each agar plate was counted and employed to calculate the viable *E. coli* concentration in the sample (N_c) based on the following equation:

$$N_c = \frac{N \times V_i \times F}{Q \times t \times V_p \times 10^{-3}}$$

where

N_c = viable *E. coli* concentration within the sample (CFU/m^3)

N = the number of colonies on an agar plate after incubation (CFU)

F = the dilution factor used to prepare the suspension for the agar plate

Q = sampling flowrate (L/min)

t = sampling time (min)

V_i = total volume of impinger and rinse liquids (ml)

V_p = the volume of diluted or undiluted suspension used for each agar plate (ml)

An Aerosizer (Model 8000, API, Inc., Hadley, MA, USA) was employed to measure the number concentration of *E. coli* in the test chamber (N_t). The Aerosizer, which is an aerodynamic particle sizer, can measure particles exceeding 0.5 μm at an airflow rate of 6 L/min. Total recovery rate is the ratio of the viable *E. coli* concentration in the sample (N_c) to that in the test chamber (N_t).

For quality assurance and quality control procedures, the aerosol generator, impingers, collection liquid, and deionized water were sterilized in an autoclave prior to each run. The aerosol generated from deionized water without adding *E. coli* was sampled and incubated to ensure that the deionized water contained no bacteria. Only those plates with an appropriate number of colonies, normally between 30 and 300, were counted. Notably, any two partially overlapped colonies were counted as two colonies. Among all samples, the number of partially overlapped colonies was about 3% of the total number thereof. Each plate was counted three times to improve accuracy. To determine the *E. coli* concentration in one sample, several agar plates were counted and the totals were averaged. Moreover, in order to reduce the standard error to less than 5%, a minimal total of over 400 colonies were employed to determine the concentration in one sample. Within each set of experimental conditions, data that did not fall in the 95% confidence interval of the mean value were rejected.

Total recovery depends on the viability of the prepared bacterial strain, which varies with preparation process. Since the *E. coli* bacteria were prepared identically herein, their viability should remain the same from run to run.

3. Results and Discussion

The geometric mean aerodynamic diameter of *E. coli* aerosol particles that were generated herein ranged from 0.8 to 0.9 μm, and had a geometric standard deviation of 1.19. Table 1 presents the *E. coli* concentration in liquid suspension in the nebulizer and in the test chamber. The bacterial concentrations employed herein were of a medium level found in contaminated environments. During a run, water in the aerosol generator evaporated slightly, resulting in a small increase in bacterial concentrations in both the liquid suspension and the test chamber. Figures 1 and 2 indicate that the total recovery of single impingers, of both bent and straight inlets, increased with sampling flowrate at all sampling times tested. The results also indicate that an increase in impaction velocity produced an increase in collection efficiency. Bent and straight inlet impingers did not markedly differ in terms of total recovery because the geometric mean aerodynamic diameter of the bacterial particles was smaller

Table 1 Comparison of *E.coli* concentrations (C_{susp}) in liquid suspension in the nebulizer and *E.coli* concentrations (C_o) in the test chamber measured before and after sampling

		Before Sampling	After Sampling
C_{susp}	Range (10^6 CFU/ml)	1.98-51.1	3.88-58.4
	Mean (10^6 CFU/ml)	25.1	25.2
	Standard deviation (10^6 CFU/ml)	13.2	15.7
	Coefficient of variation	0.52	0.63
	Sample number	49	49
C_o	Range (10^6 particles/m^3)	1.03-7.93	1.14-9.96
	Mean (10^6 particles/m^3)	3.4	4.59
	Standard deviation (10^6 particles/m^3)	1.65	2.54
	Coefficient of variation	0.49	0.55
	Sample number	49	49

than 1 μm and the air velocity at the inlet was only 2.17 m/s for the maximum sampling flowrate tested.

Figures 3 and 4 show that for all the sampling flowrates tested, the total recovery of single impingers attained a maximum at 2.5 minutes for both inlets. The maximum occurred probably owing to two competing processes: the killing of collected bacteria by incoming jets and their dispersion to regions outside of the impact zone. The stress on bacteria occurs primarily when the high velocity air stream collides with collection liquid in the impact zone (see, for example, Terzieva *et al.*, 1996). During short sampling times, a higher fraction of collected bacteria are killed in the impact zone, as they do not have sufficient time to disperse. However, as the sampling time is increased, the bacteria have sufficient time to escape the impact zone and therefore a higher fraction of them survive. During longer sampling times, dispersion decreases as the bacteria in collection liquid are relatively well mixed. Consequently, a higher fraction of bacteria are killed at longer sampling times, which thereby decreases the total recovery rate.

Figures 5 and 6 show the total recovery of the first and second impinger in two impingers that were connected in series at a sampling time of 5

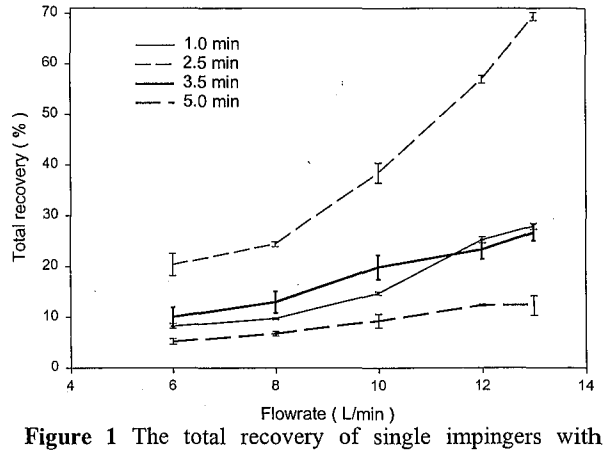

Figure 1 The total recovery of single impingers with curved inlet at various flowrates and sampling times. Each data point and error bar represent the mean and the standard deviation of 7-9 samples.

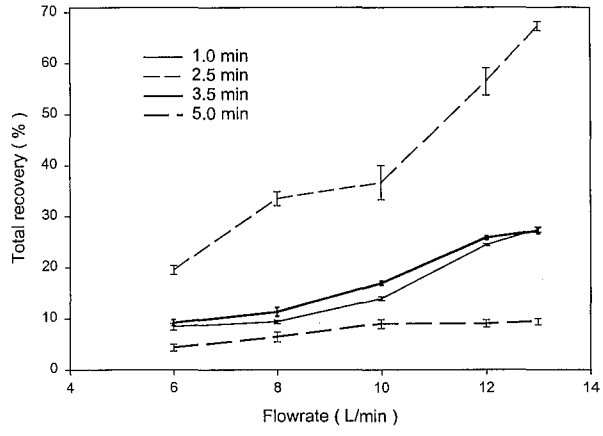

Figure 2 The total recovery of single impingers with straight inlet at various flowrates and sampling times. Each data point and error bar represent the mean and the standard deviation of 5-8 samples.

minutes and flow rates of 6, 8 and 10 L/min, and Figure 7 presents the combined total recovery of two impingers connected in series. The two impingers in series were either two bent inlets or one straight with one bent inlet. The total recovery of the first and second impinger, and the combined total recovery of the two impingers all increased with sampling flowrate. However, the second impinger in the test that had one straight and one bent inlet connected in series did not. The total recovery of the second impinger was 47-73% of that of the first impinger.

The results for single impingers depicated in Figs. 1 and 2 indicated that the total recovery rates were the lowest at the 5 minutes sampling

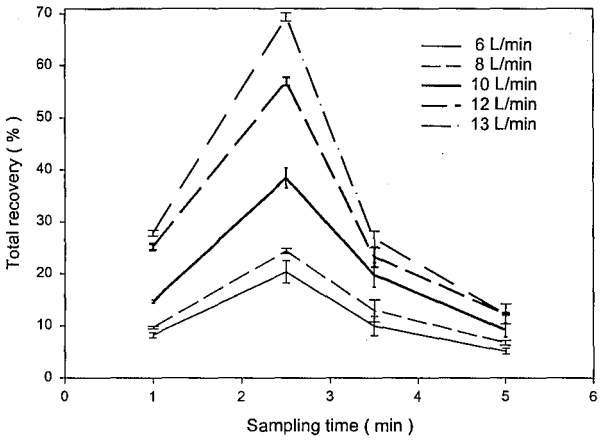

Figure 3 The total recovery of single impingers with curved inlet at various sampling times and flowrates. Each data point and error bar represent the mean and the standard deviation of 7-9 samples.

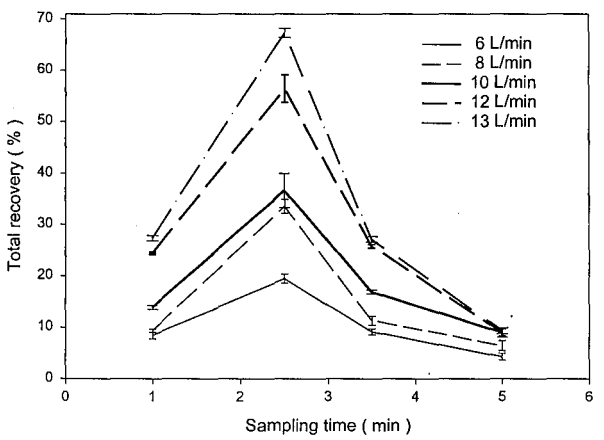

Figure 4 The total recovery of single impingers with straight inlet at various sampling times and flowrates. Each data point and error bar represent the mean and the standard deviation of 5-8 samples.

time. Therefore, we investigated the total recovery rate at this sampling time of two impingers connected in series to examine the possibility of collecting additional bacteria in the second impinger. To further examine whether the bacteria collected in the second impinger were mainly those that were not collected in the first impinger, the following experiment was conducted:

An impinger that had a bent inlet was employed initially to sample bacteria at 10 L/min for 2.5 minutes. Following sampling, 1 ml of the impinger liquid was removed for incubation and the remaining liquid was replenished with 1 ml of fresh collection liquid. A second impinger that had a bent inlet, which contained fresh collection liquid, was connected with the first impinger and then a bacteria-free aerosol stream was passed

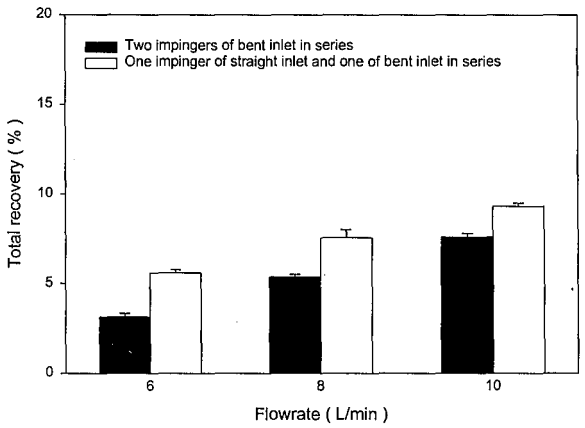

Figure 5 The total recovery of the first impinger in two impingers connected in series. Each broad bar represents the mean of 6 samples, and the error bar represents the standard deviation. (Sampling time = 5 min)

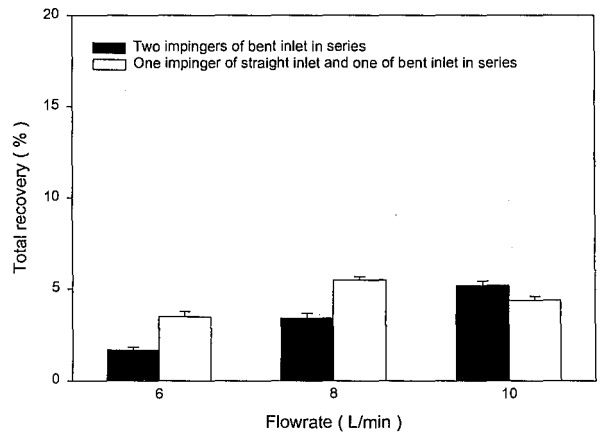

Figure 6 The total recovery of the second impinger in two impingers connected in series. Each broad bar represents the mean of 6 samples, and the error bar represents the standard deviation. (Sampling time = 5 min)

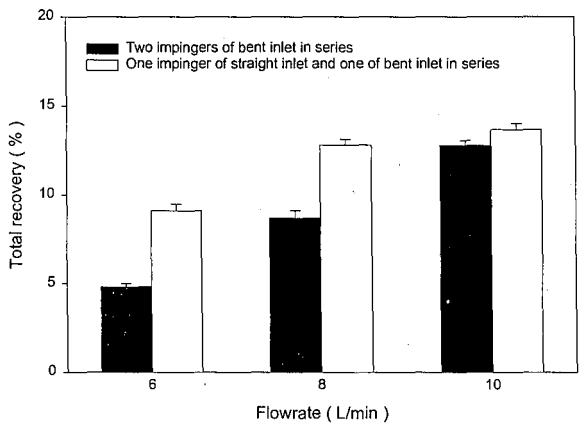

Figure 7 The combined total recovery of two impingers connected in series. Each broad bar represents the mean of 6 samples, and the error bar represents the standard deviation. (Sampling time = 5 min)

through the two impingers connected in series at 10 L/min for 2.5 minutes. Following sampling, the collection liquids in the two impingers were removed and incubated separately. The results indicated that only a limited number of bacteri were collected in the second impinger, indicating that re-entrainment of bacteria collected in the first impinger was negligible.

In Figs. 1 to 7, the total recovery rates were represented by the mean values of at least 5 samples for which the coefficient of variation was in the range of 0.01 to 0.19.

4. Conclusions

For the sampling flowrates and times tested, inlet tube shape had no marked effect on the total recovery rate of single AGI-30 impingers for *E. coli*. The total recovery increased with sampling flowrate and attained a maximum at 13 L/min for both curved and straight inlet tubes. Within all the tested flowrates, the total recovery of single impingers achieved a maximum at the 2.5 minutes sampling time. Regardless of the type of inlet tube, the combined total recovery of two impingers connected in series increased with sampling flowrate. The total recovery of the second impinger was 47-73% of the total recovery of the first one. Connecting two impingers in series increased total recovery, but also increased pressure drop.

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