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Compressed air is used in a number of processes in the food industry. It is used as an ingredient in whipped products such as ice cream, to slice or cut soft products and to open packages before filling of product. Currently, food manufacturers are under pressure to validate the safety of all ingredients or processes for regulatory compliance, but unfortunately, there is currently no standard method to evaluate the microbial content of compressed air.

The challenge to sampling compressed air is it must be decompressed prior to sampling. The Andersen One Stage viable particle sizing sampler is an impactor developed with the National Institute for Occupational Safety and Health (NIOSH) and is an approved method for bioaerosol sampling of non-compressed air by the Environmental Protection Agency (EPA), Occupational Safety and Health Association (OSHA) and the Food and Drug Administration (FDA)[2]. For this portion of the study we used the SKC BioStage, an Andersen single stage sampler with 400 holes and a cut off diameter of 0.65µm and designed to sample aerosols of bacteria from air at atmospheric pressures[1]. By comparison, the CAMTU was developed by Parker Hannifin for direct testing of compressed air[2]. Both CAMTU units collect

Figure 1. SKC single stage particle sampler. An agar plate sits in the inside of the unit. The device is attached to a calibrated vacuum pump which pulls air through the pin point holes onto the petri dish at a rate of 0.9994 CF/min (28.3 L/min).

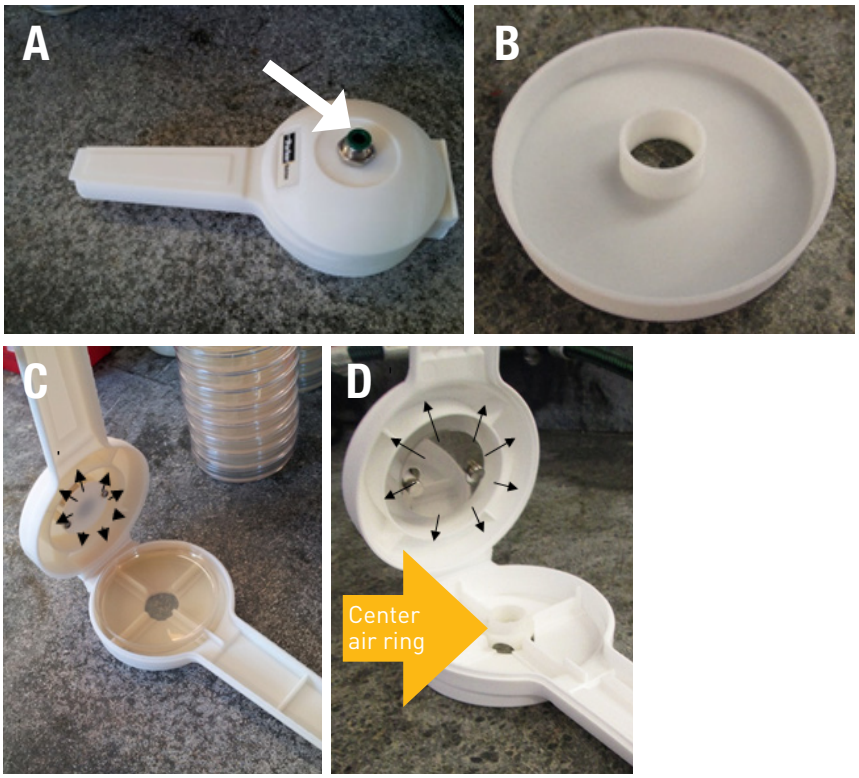

Figure 2. CAMTU Device. The outside structure (A) and the interior of CAMTU and CAMTU 2 (C and D). The closed device arrow shows the inlet (A), and the open devise shown with arrows indicating incoming air flow (C and D). CAMTU2 (D) has a center air ring to accommodate a plate with a central opening (Fig 2 B). Air exits device through channels located below the petri dish for the CAMTU and for the CAMTU 2, air exits through the center hole and similar channels along the bottom. For this study a pressure of 40 psig and an air flow rate of 1.6 CF/min were adjusted using an adjustable pressure regulator and a 0.070 inch orifice.

bacteria due to positive pressure from the compressed air pushing the bacteria onto the plate. The level of impact stress has been shown to effect microbial recovery on agar and be dependent upon the impaction velocity of the cells into the agar as well as the design and operating parameters[3]. For this reason, it's important to characterize the recovery efficiency of the CAMTU against a standard method such as the Andersen sampler.

The purpose of this study was to compare the newly designed CAMTU2 to the previous version (CAMTU), and to compare the recovery of bacteria in compressed air to the 400 hole SKC single stage impactor.

Materials and Methods

Bacterial Cultures. The Gram positive, non-sporeforming bacteria Micrococcus luteus ATCC 4698 was used as a model organism for this study[3]. Each month, tryptic soy agar (TSA) slants were inoculated from frozen stocks and incubated at 32°C for 18 h. These working culture slants were stored at 4°C. Broth cultures for each experiment were prepared by inoculating a loopful of working culture into 50 ml tryptic soy broth (TSB) and grown with agitation (200 rpm) for 18 h at 32°C. Initial cell numbers in the overnight culture were determined by dilution and spiral plating (Spiral Biotech1) onto TSA agar and incubated overnight at 32°C. Cell numbers were determined using automated plate counting (Q-count, Spiral Biotech).

Nebulizer cleaning procedure. After each nebulization sampling, the nebulizer was sanitized and washed by submerging in 70% ethanol, sonicated cleaning (Fisher Bransonic Ultrasonic cleaner) in warm water containing laboratory detergent (Alconox) in a for 5 minutes, rinse in running distilled water, sanitation by immersion in 70% ethanol and a final submersion into sterile distilled water.

Sampling with BioStage Impactors. Sterile TSA plates with a volume of 45ml of agar were placed into bleach sanitized Biostage impactor units, and the system was set up as shown in Figure 4. Vacuum pumps were calibrated against a calibrated rotameter (SKC) at the beginning of each research session. For each test, the compressed air was turned on and the system (air, nebulizer) was run for 1 min with a measured flow rate on 1.5 L/sec, after which the vacuum pumps attached to the BioStage, which collect samples at the rate of 28.3 L/min. Impactors would be manually started, and then turned off at the desired time using a timed switch after 64 or 128 seconds, resulting in sampling of 30 or 60 L of air, respectively. Two units were run simultaneously, to give duplicates for each sampling time. The air pressure and flow rates were recorded for each run. After sampling, agar plates were removed, and the impactors and sample box were wiped down with freshly prepared 500 ppm hypochlorite, and fresh plates were added. Fresh bacterial solution was used for the next sampling run. Agar plates for the BioStage Impactors contained 45 ml TSA. Plates were incubated at 32°C for 24h. The colony numbers in the initial solution and after nebulization were determined using automated plate counting system. In order to take into account the possibility of collecting multiple particles through a single hole, cell numbers on each plate were adjusted using the positive-hole correction table for a 400-hole impactor[4] and then adjusted to CFU/liter air samples.

Sampling with CAMTU. Sterile TSA plates were placed into ethanol sanitized CAMTU. The CAMTU setup as the same as the BioStage Impactor units, except rather than decompressing the air in the box, the compressed air was directly attached to the CAMTU. CAMTU plates for this study contained 45ml TSA while the "donut" shaped CAMTU2 plates contained 43ml TSA. The flow rate into CAMTU was 3.2 CF/min (1.5 L/sec). In order to maintain equal amounts of nebulization time, bacterial solutions were nebulized into sampling box for a set amount of time, nebulization was stopped and CAMTU was hooked up and sampled for a set amount of time to obtain similar volumes of air on to those sampled with the Biostage Impactor and were performed for 20 or 40 seconds, to sample 30 or 60 L of air, respectively. After sampling, agar plates were removed and the CAMTU was wiped down with 200 PPM NaHOCl, and fresh plates were added. When multiple volumes of air were sampled with the CAMTU devices, a single bacterial sample was used and new plates were added for each sequential sample (nebulize into box for 92 sec, hook up CAMTU with plate, sample 20 sec, remove CAMTU and clean, nebulize 24 sec, hookup CAMTU with fresh plate and sample for 40 sec) . The total amount of nebulization time and sampling time can be seen in Table 1. A single CAMTU unit was used and each sampling time was performed in triplicate, with fresh bacterial solution in the nebulizer for each run. Plates were incubated and counted and CFU/Liter air sampled was calculated.

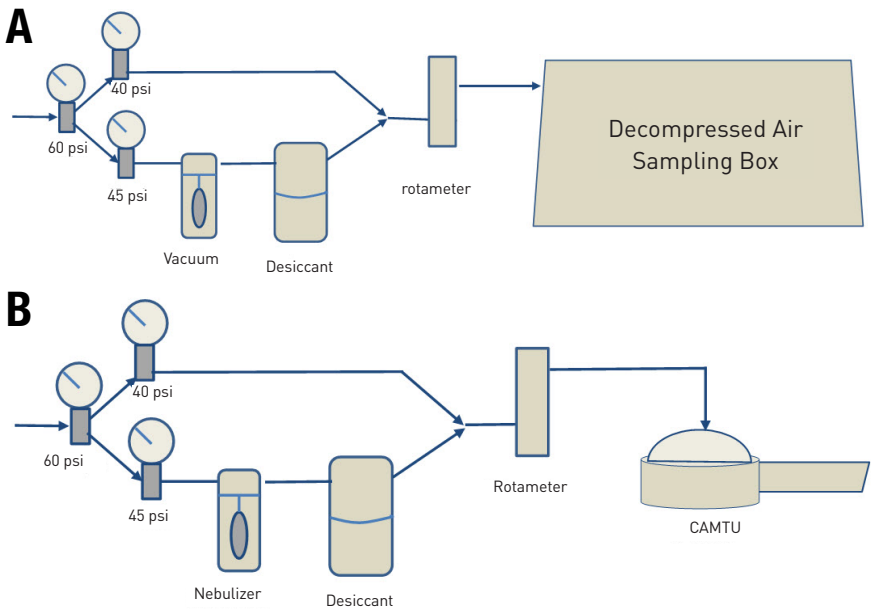

Figure 3. Sampling Set up for experiments. A) Sampling set up for BioStage air sampling. Two BioStage samplers were placed in the box and air was sampled the rate of 28.3 L/min using calibrated vacuum pumps. B) Step up for CAMTU Compressed air sampling. Compressed air was decompressed within the CAMTU device and directly impacted upon agar plates. For both set-ups, a flow rate of 1.5L/sec (3.2 SCFM) was measured using a rotameter.

Direct Comparison of CAMTU and CAMTU2. Initially, two sampling volumes (30 L and 60L) of air were tested for recovery of bacteria from compressed air (Table 2). In this study, a single concentration of bacteria was added to the nebulizer (3.32 x 107 CFU/ml). The data from each plate of the Biostage sampler was adjusted using the positive-hole correction table for a 400-hole impactor[4]. The results in Table 2 show that the CAMTU2, despite having a smaller surface area due to the center hole, there was a higher recovery of airbone bacteria than the CAMTU standard plates.

The recovery of bacterial colonies on the CAMTU2 plates was uniform across the agar surface[3b]. From this data, we concluded that the CAMTU2 recovery of bacteria on "donut plates" from compressed air is equal or better than the recovery on the traditional plates of the CAMTU. This is likely due to the air movement within the CAMTU units. The Biostage bacterial recovery was 16-17% higher than the CAMTU2 recovery. However, for both methods (CAMTU2 and BioStage),

Results and Discussion

Sample (air volume)	Sampling Time during Nebulization (sec)	Pre-sampling Nebulization time (sec)	Total Nebulization time of culture (sec)
	Andersen sampling (both initial and recent testing)		
Biostage (30L)	64	60	124
Biostage (60L)	128	60	188
	CAMTU testing		
CAMTU (30L)	20	104	124
CAMTU (60L)	40	148	188

Table 1. Nebulization time during sampling

the recovery numbers were at the high end of accuracy for agar plating methods. In general, data from plate counts is most accurate when numbers are in the range of 25 – 250 colonies on a plate[1]. In addition, for all three testing methods, there was a lower recovery per liter of air, when 60 L of air were selected. We have seen this trend time and time again. We now believe it is likely that this is due to nebulizer clogging as the units ran for additional time. Thus the longer the units run, the lower the efficiency of bacterial nebulization. *In order to prevent nebulizer clogging issues, bacterial recovery was tested only at the 30L level in later tests and the concentration of bacterial cells in the nebulizer was varied to determine a dose response of the sampling units.*

Dose Response Comparison of CAMTU2 to Biostage air sampling. In this set of experiments, bacterial cultures were diluted to create a variety of initial bacterial concentrations within the nebulizer. Four replicates of each bacterial concentration were sampled. Air sampling volume was kept constant at 30L. The Biostage values were adjusted using a correction table and the results are shown in Figure 4. In general, there were variable amounts of bacteria recovered by both sampling units, however, replicate data points are generally within 0.5 log CFU of each other. This variability may be due to the inconsistencies of the nebulization system or the capture systems itself. Since we do not have a method to count particles within the nebulization stream, there is no way to know the source of the system variation. *However, this data shows there is a definite dose response from both methods, so that I'm comfortable saying that the unit can be used for a semi-quantitative assessment.*

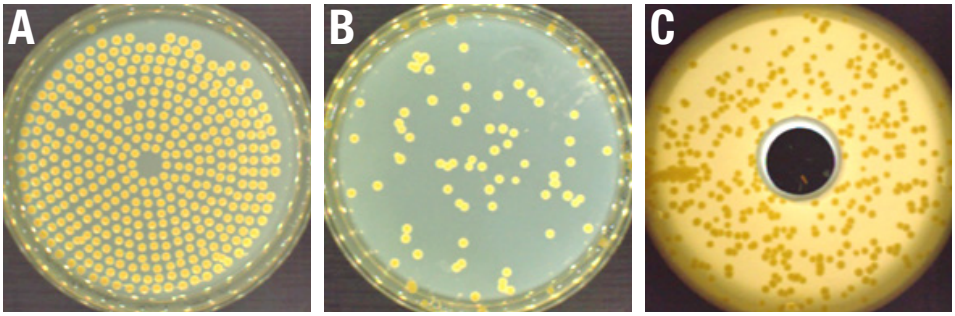

Figure 4. Micrococcus luteus colonies growing on agar plates after being recovered from air. A) Biostage recovery, the pattern is reflective of the hole pattern on the lid of the sampler. B) CAMTU and C) CAMTU2.

	CAMTU		CAMTU2		BioStage 400 hole impactor		
	30L[1]						
Rep	CFU/plate	CFU/L air	CFU/plate	CFU/L air	CFU/plate	Adj value	CFU/L air
1	133.00	4.43	249.00	8.30	391.00	1518.00	50.60
2	137.00	4.57	250.00	8.33	390.00	1475.00	49.17
3	80.00	2.67	297.00	9.90	395.00	1754.00	58.47
Avg value	116.67	3.89	265.33	8.84	392.00	1582.33	52.74
St dev	25.98	0.87	22.40	0.75	2.16	122.65	4.09
	60L[1]						
Rep	CFU/plate	CFU/L air	CFU/plate	CFU/L air	CFU/plate	Adj value	CFU/L air
1	88.00	1.47	325.00	5.42	385.00	1313.00	21.88
2	163.00	2.72	283.00	4.72	398.00	2127.00	35.45
3	179.00	2.98	275.00	4.58	398.00	2127.00	35.45
Avg value	143.33	2.39	294.33	4.91	393.67	1855.67	30.93
St dev	39.67	0.66	21.93	0.37	6.13	383.72	6.40

Table 2. Comparison of Recovery on CAMTU and CAMTU II to Biostage impactor

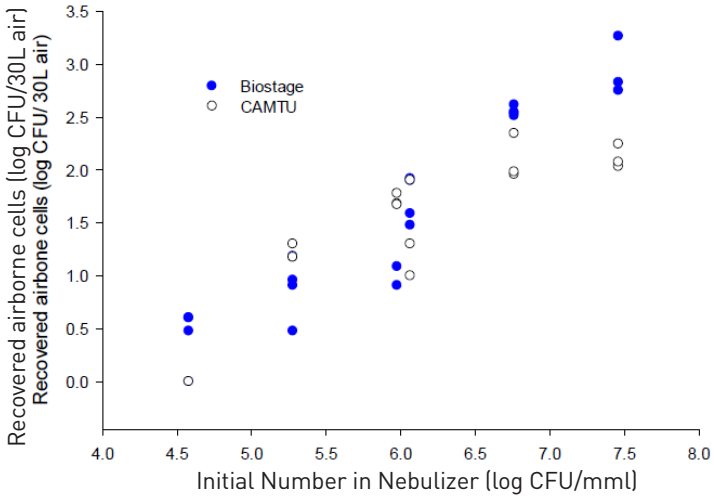

Figure 5. Dose response curve of CAMTU II in comparison to the Biostage. Bacterial cultures were diluted to create a variety of concentrations within the nebulizer. A total of 30L of air was sampled for each data point. Biostage values were adjusted using a correction table.

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