The Distribution of Airborne Mesophilic Bacteria, Yeasts and Molds in Beet Sugar Factories[1]

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The control of bacteria, yeasts and molds in finished crystalline sucrose is a problem faced by every producer of this very marketable chemical. Due to the ubiquitous nature of these microorganisms, their sources in finished sugar may be manifold, and the air in and about the factories has always been suspect. For this reason most, if not all, sugar manufacturing concerns go to great lengths to filter the air which contacts the finished product. Such filtering mechanisms may include banks of treated fiber glass filament furnace filters and Precipitrons or the furnace filters alone. Drying the wet granules immediately after washing requires a large volume of clean, warmed air. Forced circulation of air over sugar in bulk storage is apparently necessary to minimize moisture condensation in the silos. The air in both cases must be filtered to maintain as low a microflora contamination as possible.

Very little quantitative data are available which describe the contribution of air to the contamination of finished granulated sugar. This paper is a report of a preliminary study of the air in and about two beet sugar factories. Both were showing occasional high microorganism counts in finished sugar.

Material and Methods

Air Sampling

The air was sampled by the use of the Andersen Sampler (1)[3]. A diagramatic sketch of this instrument is shown in Figure 1. The sampler is a unique cascade type air sampling instrument. Air drawn through the sampler at a given rate, i.e., 1 cu foot per minute, passes through the six stages of the sampler and at each stage is impinged onto the surface of a nutrient agar plate containing medium prepared to grow the type of specific organism sought. There are 400 holes in each stage cover but from stage to stage, proceeding from 1 through 6, the holes become smaller, thus serving to increase markedly the velocity at which the air and particles are impinged upon the agar plate immediately below each cover. This velocity increase at each stage separates the particles suspended in the air into different sizes according to the mass of each particle.

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[3] Numbers in parentheses refer to literature cited.

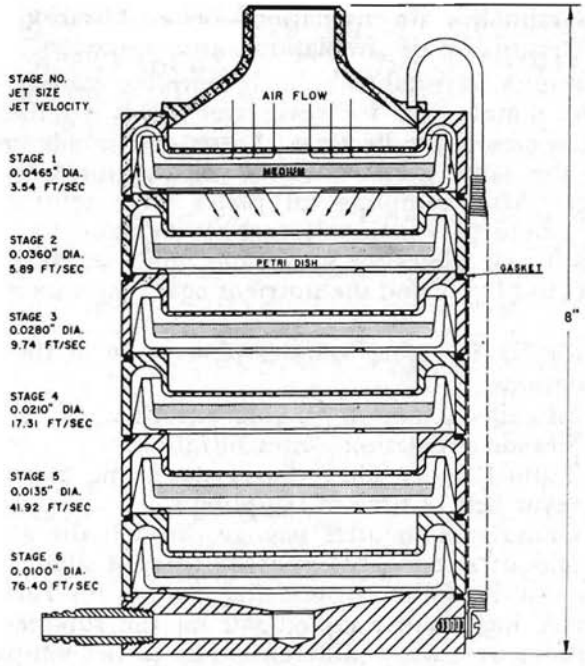

Figure 1.—Andersen Sampler.

Only the very large particles are deposited at stage 1 and at each stage the deposited particles are smaller until at the final stage, (No. 6) only particles of 1 micron or less impinge upon the surface of the growth media. Larger particles are distributed according to size among the intervening 5 plates. Should a particle be carrying a viable mold or yeast spore, or a mesophilic bacterium then a colony will grow to visible size in a few hours. Colonies may be counted and the number of microorganisms per cubic foot of air estimated.

Media

For the detection of yeasts and molds, BBL[4] mycophil agar plates were prepared with the pH of the agar adjusted to 4.5.

For detection of mesophilic bacteria, BBL nutrient agar adjusted to pH 7.0 was used in the plates.

Sampling procedure

At Factory A, 5 sampling stations were established as follows:

A. Silo air circulation—before filtration.
B. Silo air circulation—after filtration.
C. Tunnel under the silos. (Sugar was being moved on a conveyor belt at time of sampling.)

[4] BBL - Baltimore Biological Laboratory

D. Granulator air circulation—before filtration.
E. Granulator air circulation—after filtration.

Four samples were taken at each sampling station, a 5 min. and 10 min. sample each for yeasts and molds together and the same for the mesophilic bacteria. Yeasts and molds may be detected on the same plates and are distinguished by colonial morphology. After sampling all plates were returned to the laboratory where they were allowed to incubate for 72 hours; the mycophil agar plates for yeasts and molds at room temperature (22° C to 24° C), and the nutrient agar plates for mesophiles at 35° C.

At Factory B, 5-minute samples were taken at the following sampling stations:

A. Silo air circulation—before filtration.
B. Silo air circulation—after filtration.
C. Tunnel under silos. (Sugar was being moved on conveyor belt at time of sampling.)
D. Granulator air after passage through the granulator.
E. Silo air system. Air in area at top of silos.

The procedures followed were the same as for Factory A.

Factory A was again sampled late in the summer. At this time conditions were much different. The earlier samplings were taken during the manufacturing cycle. At the second sampling the plant was not in operation; many of the silos were empty and sugar movement was limited to transport by conveyor belt to bulk cars, to the classifying room, or recirculation to another of the large silos. Except for a slight change in the sampling stations, the procedures were exactly the same as described above:

A. Silo air circulation—before filtration.
B. Silo air circulation—after filtration.
C. Tunnel under silos. (Sugar was being moved on conveyor belt at time of sampling.
D. Classifying room. (Sugar dust was being blown about at the time of sampling.)

Results

First Sampling—Factory A. The results of the sampling of the air for molds, yeasts, and bacteria at the various sampling stations are presented in Table 1. The term "total" represents the total number of organisms from all six stages of the sampler for each sample. The totals of the 5-minute and 10-minute samples agreed well enough that the microorganisms in each class per cubic foot of air were calculated by taking the total number of organisms of both the 5- and 10-minute samplings and dividing by 15. A comparative summation of these results is presented in Table 1.

Table 1.—Microorganisms per cubic foot of air at each sampling station. Plant A.—First sampling.

		Mesophiles	Molds	Yeasts
A.	Silo air circulation before filters	5.2	13.6	4.9
B.	Silo air circulation after filters	1.0	3.5	0.5
C.	Silo—tunnel air	3.0	25.0	8.7
D.	Granulator air before filtration	22.2	5.2	5.7
E.	Granulator air after filtration and Precipitron	6.4	.3	.2

Table 2.—Microorganisms per cubic foot of air at each sampling station—Plant B.

		Mesophiles	Molds	Yeasts
A.	Silo air circulation before filters	36.6	6	.4
B.	Silo air circulation after filters	6.4	3.2	0
C.	Tunnel under silos	10.0	1.4	.4
D.	Granulator air after passage through the granulator	23.8	1.0	0
E.	Silo air system—air in area top of silo	4.6	.4	.6

Table 3.—Microorganisms per cubic foot of air at each sampling station. Plant A. Second sampling.

		Mesophiles	Molds	Yeasts
A.	Silo air circulation before filters	75.6	13	0.8
B.	Silo air circulation after filters	10.6	5	0.2
C.	Tunnel under silos	(Not sampled)	1.4	Neg.
D.	Classifying room	52.4	18	2

Factory B. A summary of the results of sampling of air in Factory B is presented in Table 2. Again the total colonies of all stages of the Andersen Sampler were utilized to arrive at the number of organisms per cubic foot of air.

Second Sampling—Factory A. Table 3 presents the results of air sampling of stations of Factory A late the following summer.

Discussion and Conclusions

Note that the highest concentration of mold spores in the first sampling, Factory A, was found in the tunnel air where the finished sugar was being transported on an endless belt to bulk cars. The belt had no protective cover. The contribution of this situation to the mold count of the sugar in the cars is unknown, but poses a potential addition of mold and yeast spores

to sugar on the belt. The tunnel air was laden with sugar dust and accounts for the large number of spores in the air.

The yeast count of 8.7 per cubic foot of air was surprisingly low. The same plates are used for the mold and yeast count, and mycophil agar adjusted to pH 4.5 was the medium used. Two conditions may have contributed to the low yeast count; i.e., the mold count was very high and it is suspected that the larger mold colonies not only hid many yeast colonies, but may have suppressed the growth of the yeasts and the low pH may have tended to inhibit the initiation of yeast growth.

The mesophile count was highest in the outside air as it was pumped into the granulator. Fortunately the bank of furnace filters and the Precipitron effectively remove these bacteria from the air supply to the granulator as indicated by the reduction of mesophilic bacteria from 22.2 to 6.4 per cu ft air. The few organisms showing on the plates probably were carried into the chamber between the Precipitron and the filter bank when the door was opened to gain entry with the sampler. There was a high negative pressure within the area between the two filters. The results of these tests certainly give one confidence in the Precipitron and fiber glass filtration system.

The fiber glass furnace filters used in the bulk silo circulation system effectively removed 3/4 of the mold spores, 9/10 of the yeasts, and 4/5 of the mesophilic bacteria. This is a fair reduction, but still leaves room for improvement of this filtering system.

The bulk storage air of Factory B was not nearly as contaminated with yeasts, 0.4 per cu ft, and molds, 6 per cu ft, as that of Factory A. In contrast, the mesophilic count was relatively high with 36.6 organisms per cu ft of air. Factory B was having a problem of a high mesophilic bacteria count in finished sugar at the time these air samples were taken. Also the number of bacteria per cubic foot of air sampled after passing through the granulator was shown to be high at 24 bacteria per cu ft. This air had been filtered and passed through a Precipitron before entering the granulator. Our experience at Plant A had indicated that air filtered in this manner was practically free of microorganisms, therefore the increase to 24 bacteria per cubic foot of air was a reflection of the high count in the newly produced sugar. It is also suggested that the high mesophilic count of the bulk silo air before filtration owes its origin to this same source. Thus a vicious cycle appears. The contaminated freshly produced sugar contaminates the air of the plant which in turn may reintroduce microorganisms into the manufacturing process, which then show up in the granulated sugar.

At the second sampling of Plant A, yeasts and molds were comparatively few in number, but the mesophilic bacteria counts were very high. In comparison with the first sampling carried out during the sugar campaign, the mold count in the silo air circulation system was about the same, but less in the tunnel beneath the silos. The number of mesophilic bacteria was much higher in the bulk silo circulation. The air in the classifying room was not sampled at the earlier sampling, but showed a high count at this time. It is interesting to note that the mesophilic counts routinely carried out on sugar being shipped from Factory A were high at the time of this sampling, and the count of mesophiles in the air circulation system before filtration reflected the count in the granulated sugar.

Summary

1. The mold count is usually high in air carrying large amounts of sugar dust as seen in the bulk silo tunnels. This is especially true where air is rapidly circulated by large fans, and the dust is "swept" off the top of the sugar. The circulation also tends to keep the dust stirred up throughout the bulk storage areas.
2. The spun glass filters (furnace filters) placed in the air circulation path remove a rather large portion of microorganisms from the air by removal of dust particles. However, the efficiency of these filters could and should be increased.
3. The Precipitron in conjunction with the spun glass filters, efficiently removes most bacteria, yeasts, and molds from air being forced through the granulator.
4. The outside air around Factory A carried a greater percentage of mesophilic bacteria than yeasts and molds. The yeasts and molds were found most often in sugar dust laden air.
5. It is apparent that a high mesophilic bacteria count in finished granulated sugar is reflected in the mesophilic count of air being circulated over that sugar.

Literature Cited

(1) ANDERSEN, ARIEL A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. of Bact. 76 (5): 471-484.