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Weighing Technique for Determining Bacterial Dry Mass Based on Rate of Moisture Uptake

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Weights were recorded at 30-s intervals timed from initial exposure of dried cells to atmosphere. Linear regressions of data over 3 min gave moisture-free dry mass estimates.

One convenient way of collecting bacteria for drying and weighing is on membrane filters. The bacteria so collected are easily manipulated, and their mass in relation to the substrate is relatively high. However, the dried cells are hygroscopic and when exposed to the atmosphere during weighing take up moisture, which becomes a source of error in the mass determination. To avoid this problem and to take full advantage of membrane filters, the bacteria should be weighed in a completely dry atmosphere, which could be costly to achieve. Practical methods suggested to avoid this error include weighing on a high-speed balance and weighing in an enclosed pig (1). Neither method is satisfactory because even with a high-speed balance the cells absorb moisture, albeit a small amount, before the mass is read, and the pig suffers the disadvantages associated with determining a small mass relative to a large mass (1).

One approach to the problem may be to accept the hygroscopic nature of bacteria and to take this into account when weighing. To do this, we must know the manner in which moisture uptake occurs. Moisture uptake is controlled by the affinity for water absorption of the many hygroscopic substances in bacterial cells, and by relative humidity. The rate of uptake depends on relative humidity and diffusion of air to the cells. Thus, a plot of uptake against time should be curved and reach a maximum at which point moisture uptake matches moisture loss.

In this note, I present data on moisture uptake of bacteria collected on membrane filters and describe a simple method for determining dry mass even at high relative humidity.

Four cultures were used, *Streptococcus cremoris* MLI, *Lactobacillus bulgaricus* LBI, *Escherichia coli* K-12, and a strain of *Pseudomonas fluorescens*. The first two cultures were grown in M17 broth (2) and MRS broth (Oxoid), respectively, and the others were grown in PYE (peptone, 10 g/liter; yeast extract, 5 g/liter; sodium chloride, 5 g/liter) broth. All broths were prefiltered through membrane filters (Sartorius; pore size, 0.45 μm) before use.

After growth, measured volumes of the cultures were centrifuged and washed twice with ice-cold saline, and the bacteria were filtered onto preweighed membrane filters (Gelman DM 450; pore size, 0.45 μm; 47 mm), where they were rinsed with ice-cold filtered demineralized water. The volumes of cultures filtered were 4× 100 ml of *S. cremoris*, 2× 100 ml each of *L. bulgaricus* and *E. coli*, and 2× 10 ml of *P. fluorescens*.

After preliminary drying on filter paper on the bench, the loaded membrane filters were put into small petri dishes and dried at 70°C under vacuum. The lids were placed on the petri dishes when moving the filters from the oven to the desiccator.

Filters were weighed by the following procedure. A petri dish was transferred from the dessicator to the bench. The petri dish lid was taken off, and simultaneously a stopwatch was started. The filter was then placed quickly on the pan of a five-place balance (Mettler H54), and the balance was released. The mass was then recorded as soon as the balance settled (usually 30 to 45 s on the stopwatch) and then at 30-s intervals timed from the starting of the stopwatch. Indicated masses were recorded until 10 min had elapsed. The relative humidity of the air in the building was >90%.

The average masses per filter of dried cells of *S. cremoris*, *L. bulgaricus*, and *E. coli* were about 32.1, 26.5, and 27.3 mg, respectively. The results from one determination of each of two organisms is shown in Fig. 1. Moisture uptake rates for these organisms were very similar and did not plateau out in 10 min. At that time, the moisture content had reached 7.8, 8.6, and 9.9%, respectively, of the subsequently estimat-

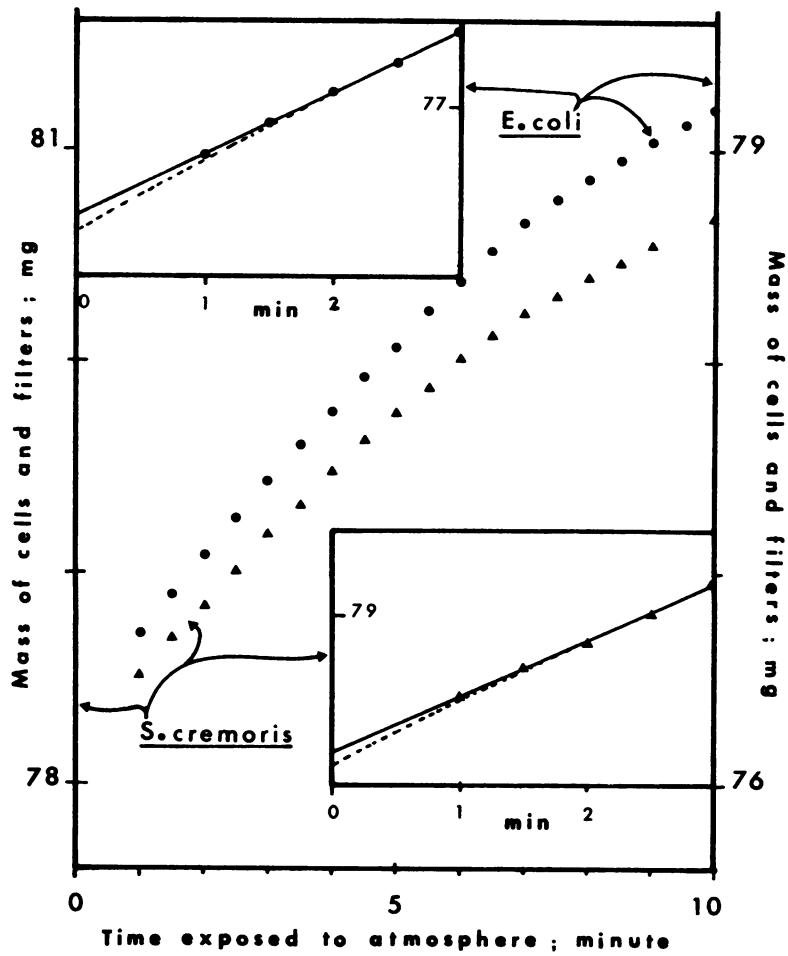

FIG. 1. Moisture uptake by bacteria in a humid atmosphere. Dried cells of *S. cremoris* and *E. coli* on membrane filters were placed on a balance pan, and the mass was recorded at 30-s intervals after exposure of the cells to the atmosphere. The inset diagrams show the 3-min linear regression line (——) and the 10-min quadratic regression line (---) for each of the organisms during the first 3 min of exposure to the atmosphere.

ed dry mass; after exposure for 24 h, the moisture contents were 12.7, 13.6, and 15.8%, respectively.

Moisture uptake by these organisms over 10 min was described by quadratic regressions whose R^2 values ranged from 0.99905 to 0.99977. These quadratic regressions were extrapolated to zero time, the time at which the cells were first exposed to the moist atmosphere. In addition, the data gathered up to 3 min were fitted to linear regressions, and the R^2 values ranged from 0.99412 to 0.99989. Again, mass at zero time was calculated from these linear regressions.

The dry mass estimates from the quadratic and linear regressions obtained from the determinations done on the three organisms above were compared with those found by reading the balance as soon as it settled (Table 1). This comparison showed that in all cases except one, the mass determined by the quadratic regression was 0.08 to 0.1 mg lower than that determined by the linear regression and that the mass obtained when the balance settled was 0.14 to 0.21 mg higher than that obtained by linear regression. These differences were statistically different ($P < 0.001$) by the F test in a two-way analysis of variance. The difference between the highest and lowest of the three estimates was about 0.9% of the dry weight.

Moisture uptake by *P. fluorescens* stopped within 3.5 to 4 min after exposure to the atmosphere, but there was only about 3 mg of cells on each filter. Dry mass estimates by any of three methods, linear regression, quadratic regression of data to 4 min, or the balance read at settle, gave similar results (Table 1).

The two regressions applied to the first three

TABLE 1. Comparison of three methods for estimating the dried mass of cells

Bacterium	Sample no.	Wt of membrane filter blank (mg)	Wt of cells (mg[a]) by method:		
			Quadratic regression	Linear regression	Balance at settle
S. cremoris	1	45.58	81.18	81.27	81.45
	2	46.40	78.11	78.19	78.40
	3	48.82	80.87	80.95	81.12
	4	48.66	80.39	80.46	80.66
L. bulgaricus	1	54.90	81.53	81.62	81.76
	2	48.88	75.06	75.16	75.33
E. coli	1	47.19	73.92	73.92	74.06
	2	48.55	76.26	76.36	76.50
P. fluorescens	1	53.62	57.28	57.31	57.27
	2	56.45	59.33	59.36	59.35

[a] Mass includes the mass of the membrane filter bank.

cultures predict different dry masses, and one must choose between them. Linear regression of data collected over 3 min is probably the best method. With it, dry mass can be found either by using a simple programmable calculator or by fitting a line by eye to the points on a graph. In addition, in all but 1 of the 10 determinations done in this experiment, the quadratic regression line passed below the 1-min point, which is closest to the zero time point, whereas the linear regression passed through this 1-min point. By using the linear regression method, the dry mass determined was about 0.6% lower than the reading from the balance in high relative humidity.

The method should be useful for reducing the error of an estimate of dry mass of bacteria, particularly in a situation of high humidity, in which moisture uptake is rapid.

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