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Buoyant Densities and Dry-Matter Contents of Microorganisms: Conversion of a Measured Biovolume into Biomass

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Several isolates of bacteria and fungi from soil, together with cells released directly from soil, were studied with respect to buoyant density and dry weight. The specific volume (cubic centimeters per gram) of wet cells as measured in density gradients of colloidal silica was correlated with the percent dry weight of the cells and found to be in general agreement with calculations based on the partial specific volume of major cell components. The buoyant density of pure bacterial cultures ranged from 1.035 to 1.093 g/cm^3, and their dry-matter content ranged from 12 to 33% (wt/wt). Average values proposed for the conversion of bacterial biovolume into biomass dry weight are 1.09 g/cm^3 and 30% dry matter. Fungal hyphae had buoyant densities ranging from 1.08 to 1.11 g/cm^3, and their dry-matter content ranged from 18 to 25% (wt/wt). Average values proposed for the conversion of hyphal biovolume into biomass dry weight are 1.09 g/cm^3 and 21% dry matter. Three of the bacterial isolates were found to have cell capsules. The calculated buoyant density and percent dry weight of these capsules varied from 1.029 g/cm^3 and 7% dry weight to 1.084 g/cm^3 and 44% dry weight. The majority of the fungi were found to produce large amounts of extracellular material when grown in liquid cultures. This material was not produced when the fungi were grown on either sterile spruce needles or membrane filters on an agar surface. Fungal hyphae in litter were shown to be free from extracellular materials.

Many quantitative soil microbiological studies include the measurement of the biovolume of bacteria and fungi. This biovolume estimate is normally converted into cell dry weight and nutrient contents, to be related to other processes in the soil, such as respiration, mineralization, and immobilization of nutrient elements (15).

The conversion of measured biovolume into biomass is also needed for the calibration of the more indirect methods for biomass determination, such as CO_2 respirometry after fumigation with $CHCl_3$ (12).

A microscopically determined biovolume may be converted into biomass if the density and the percent dry weight (weight/weight) of the cells are known. In the literature, widely different values for these two parameters have been assumed or measured. The density values range from 1.1 g/cm^3 (20) to 1.5 g/cm^3 (17), and the dry-matter content from 10 (10) to 20% (9). Clearly, the extremes of these values can give very different conclusions from experiments in which growth yields are calculated (8).

Because we considered averages of literature values to be rather unreliable, the present study of the relationship between microscopically determined biovolume and biomass was begun. The study also involved the determination of the buoyant density of the cells.

MATERIALS AND METHODS

Isolation and cultivation. Bacteria were isolated from an agricultural clay loam described by Uhlen (22). Pure strains were transferred to 500-ml Erlenmeyer flasks containing 200 ml of nutrient broth (1% peptone, 1% yeast extract, 0.5% glucose, 0.02% $MgSO_4 \cdot 7H_2O$, 0.0005% $MnSO_4 \cdot 4H_2O$, 0.14% K_2HPO_4, and 0.15% KH_2PO_4). The flasks were incubated at 21°C on a rotary shaker during growth. The cells were harvested after 1 to 3 days, depending on growth rate. The cells were concentrated by centrifugation (5,000 × *g* for 20 min) and fixed by suspension in 1.25% glutaraldehyde solution. After fixation, they were washed three times in distilled water. The suspensions used for further investigation contained 10^9 to 10^{10} cells per ml.

A top-driven homogenizer (Measuring & Scientific Equipment, Ltd., London) was used for suspension of the cells after centrifugation. The growth medium, the glutaraldehyde solution, and the water were filtered through membrane filters (pore size, 0.2 μm) before use.

Mycelial cultures were isolated from a spruce forest litter in the vicinity of the Agricultural University (28 km south of Oslo, Norway). The basidiomycetes were

isolated from basidiocarps growing in spruce needle litter (J. Hovland and R. A. Olsen, manuscript in preparation). Small parts of the insides of the caps or stems were incubated on malt agar. Before being transferred to the culture collection, the mycelia were checked for clamp connections and absence of contaminating organisms.

Fungal material for dry-weight studies was grown either in liquid culture in 100-ml Erlenmeyer flasks or on 47-mm sterile membrane filters (GN-6; 0.45 μm; Metricel; Gelman Sciences, Inc., Ann Arbor, Mich.) on an agar surface. We used either malt extract medium (16) or a synthetic glucose-ammonium tartrate medium (14). The flasks and the agar plates were inoculated with small pieces of a culture growing on malt extract agar.

The mycelial mats from the liquid media were harvested after 10 to 18 days, depending on the growth rate, washed thoroughly, homogenized, and used for density gradient centrifugation and dry-weight determination. Naturally occurring mycelia were collected from needle litter in spruce forests containing large amounts of fungal materials. Mycelia of different basidiomycetes grown in pure culture on milled needles were also used.

Fresh and dry weight of bacterial pellets and mycelial mats. After centrifugation for 20 min at 5,000 × *g*, bacterial pellets were spread out on a 0.2-μm polycarbonate filter (Bio-Rad Laboratories, Richmond, Calif.), and suction of 81 kPa was applied for 2 min while the material was kneaded with a spatula. The mycelial mats from the liquid cultures were harvested on a metal screen and sucked dry in the same way.

After most of the water between the cells was removed by these procedures, the percent dry weight was determined by weighing before and after drying at 105°C.

Buoyant density measurement. The buoyant density of bacterial cells and fungal hyphae was measured by isopycnic centrifugation in a self-forming gradient of colloidal silica (25). Ludox HS 40 (Du Pont Co., Wilmington, Del.) was diluted with distilled water to the specific density of 1.1 g/cm^3. The pH was adjusted to 7.0 by the addition of 0.5 N HCl under vigorous stirring.

A Sorvall SS1 centrifuge equipped with an angle head was used. Polycarbonate tubes with a total capacity of 40 ml were filled with 30 ml of Ludox solution, and centrifugation was performed at top speed, about 30,000 × *g*, for 1 h at 5°C.

Bacterial or hyphal suspensions (2 ml) were laid on top of the preformed gradients and centrifuged for 1 h at 10,000 × *g*. In each run, one of the tubes was loaded with density marker beads (DMB) (Pharmacia Fine Chemicals, Uppsala, Sweden). Estimates of the buoyant density of the cell bands were obtained by comparing their positions in the gradient with the position of the different DMB.

The DMB had to be calibrated for our experimental conditions, as they are calibrated by the producer for use in Percoll (Pharmacia Fine Chemicals) containing 0.1 M NaCl. We omitted the NaCl in our experiments because the Ludox tended to gel when NaCl was added.

Six parallel gradient tubes were produced, two of which were loaded with DMB. Small samples (0.5 ml) were taken from the positions of the different DMB in the gradients. The percent dry weight (weight/weight) of these samples was determined and used to calculate the buoyant density of the beads. The calculation was based on the experimentally determined relationship between density and the percent dry weight (weight/weight) of the Ludox solution.

Biovolume measurements. The bacterial numbers per ml of all suspensions were determined microscopically after staining with acridine orange (11). The cell volume was calculated from the cell dimensions measured in a fluorescence and phase-contrast microscope. The wet mycelia were homogenized in 10 ml of distilled water for 1 min in an MSE homogenizer. The total volumes were measured, and 5 ml was taken for dry-matter determination. The rest was used for biovolume measurement. The hyphal length and diameter were measured by epifluorescence, using fluorescein isothiocyanate (1).

Slime capsules surrounding bacterial cells and extracellular materials around fungal hyphae were made visible by negative staining with India ink and were examined by phase-contrast microscopy.

Release of cells from fresh soil. To measure the buoyant density of bacterial cells in soil, the cells were first released and separated from hyphae and soil debris by blending and low-speed centrifugation, as described by Fægri et al. (9).

Hyphae from fresh soil were provided by grinding material from the F-1 layer in spruce forest soils containing large amounts of hyphae. The material was ground in a mortar to a homogenous paste. The paste was further diluted 1:10 and laid on the density gradient.

Dry-matter determination and calculation of the percent dry matter in the cells. The percent dry weight (weight/weight) of bacterial cells and fungal hyphae in suspensions was calculated from the microscopically determined biovolume (*V*), the buoyant density of the cells (*p*) as measured in the density gradients, and the content of dry matter (*DM*) determined after drying at 105°C for 12 h. The percent dry weight (weight/weight) was calculated from the formula $(DM \times 100)/(V \times p)$.

RESULTS

Calibration of the DMB in Ludox HS 40 showed that the beads with the lowest densities had somewhat lower buoyant densities in Ludox without NaCl than in Percoll with added NaCl. However, the differences were small.

In an early experiment, bacterial and fungal cells were centrifuged in a CsCl solution with a density of 1.38 g/cm^3. The cells were found to float on top of the solution during the first 2 to 3 min of centrifugation, and then the band began to broaden into the CsCl solution; after 10 min, all the cells had sedimented to the bottom of the tube.

The buoyant density of bacterial cells which had been fixed and washed was compared with that of untreated cells taken directly from the growth medium. With capsulated isolates, the buoyant density was found to be slightly increased by fixation and washing, whereas cells without capsules were not affected by this treatment.

The dimensions and volumes of the bacterial cells and the slime capsules are shown in Table 1. Both spherical and rod-shaped cells are represented, and the cell volumes range from 0.18 to 17.6 μm^3. Three of the isolates had slime capsules, and the volume of these cells plus capsules was five to seven times larger than the volume of the corresponding naked cells.

Table 2 shows the results of the determination of different parameters measured for the bacterial isolates. The isolates seemed to fall into two distinct groups with respect to buoyant density. Four isolates had values between 1.035 and 1.048 g/cm^3, whereas the values for the remaining five isolates varied from 1.081 to 1.093 g/cm^3. Both groups contained capsulated isolates. No satellite bands were observed. The very broad bands of isolates 3 and 9 were evidently not caused by failure to reach equilibrium, as further centrifugation for 2 h at 10,000 $\times$ *g* gave the same results.

The dry weight of the bacteria without slime capsules was found to range from 25 to 38%; an exception was isolate 9, which contained only 13%.

By ignoring the presence of extracellular material, one would obtain anomalously high estimates of the dry-matter contents of capsulated cells. As shown in Table 2, the calculated percent dry weight of isolates 1, 3, and 5 would be 205, 102, and 59%, respectively. If, on the other hand, the calculation were based on the volume of cells plus slime, the percent dry weight would be 41, 15, and 12%, respectively.

It is also shown in Table 2 that the bacterial pellets, after being sucked as dry as possible on membrane filters, contained 20 to 32% dry matter.

About 40% of the bacterial cells released from soil formed a band between 1.04 and 1.12 g/cm^3, and the rest were found to sediment through the gradient together with the clay and humus material. The size distribution of the cells in the bacterial band was practically the same as that in the suspension which was loaded on the gradient.

Fungal isolates grown in liquid malt extract medium were also subjected to measurement of different parameters, necessary for the calculation of dry-matter contents based on biovolume measurements. The results are shown in Table 3.

The buoyant density for the tested fungi showed great diversity. The two *Trichoderma* spp. had buoyant densities of 1.074 and 1.081 g/cm^3, whereas the *Mortierella* sp. had a density of 1.054 g/cm^3. The buoyant densities of the different basidiomycetes varied from 1.027 to 1.077 g/cm^3 for the major band formed in the gradient centrifugation tube. For three of the tested fungi, all belonging to *Mycena*, the density gradient centrifugation showed a major band with a low average buoyant density ranging from 1.027 to 1.036 g/cm^3 and a faint satellite band with a density of about 1.105 g/cm^3. The bulk of the hyphae of these fungi was found in the low-density layer. The four other fungi, belonging to four genera of basidiomycetes, showed only one band in the density gradient, with a buoyant density ranging from 1.060 to 1.077 g/cm^3.

By microscopical examination of negatively stained material of different fungi, it was found that all basidiomycetes had extracellular material attached to the hyphae when growing in liquid culture. As indicated in Table 3, the extracellular material of the *Mycena* spp. was only loosely attached to the hyphae. In the homogenized suspensions of these fungi, one could observe hyphae without extracellular material, as well as fragments of extracellular material released from the hyphae. In suspensions of the other fungi, the extracellular material remained attached to

TABLE 1. Cell shape, size, and calculated volume of bacterial isolates and size and calculated volume of cells with slime capsules

Isolate no.	Cell shape[a]	Cell width (μm)	Cell length (μm)	Cell vol (μm^3)	Cells + Slime	
					Diam[b] (μm)	Vol (μm^3)
1	S	0.7		0.18	1.2	0.9
2	R	1.0	2.4	1.88	†[c]	
3	R	0.7	1.0	0.38	1.7	2.57
4	R	0.7	4.0	1.54	†	
5	S	1.0		0.52	1.7	2.57
6	R	0.8	2.3	1.16	†	
7	R	1.0	2.0	1.57	†	
8	R	0.7	1.0	0.38	†	
9	R	2.0	5.6	17.6	†	

[a] R, Rods; S, spherical cells.
[b] The slime capsules were spherical.
[c] †, Cells without visible slime capsules.

TABLE 2. Buoyant density of bacterial cells in Ludox gradients, dry weight per cell, calculated percent dry matter, and percent dry matter in pellets

Bacterial isolate	Limits of density in Ludox (g/cm^3)[a]	Avg density (g/cm^3)	Dry wt per cell (pg)	Calculated % dry matter[b] (wt/wt) based on		Dry matter (wt/wt) in pellets (%)
				Vol of naked cells	Vol of cell + slime	
1	1.075–1.087	1.081	0.40	205	41	29
2	1.087–1.098	1.093	0.51	25	†[c]	20
3	1.020–1.049	1.035	0.40	102	15	27
4	1.080–1.090	1.085	0.63	38	†	20
5	1.033–1.049	1.041	0.32	59	12	21
6	1.075–1.087	1.081	0.37	30	†	32
7	1.091–1.094	1.092	0.65	38	†	ND[d]
8	1.045–1.050	1.048	0.12	31	†	ND
9	1.020–1.055	1.038	2.33	13	†	ND

[a] The limit of density in Ludox for cells released from soil was 1.040 to 1.120 g/cm^3.
[b] Estimated from microscopical counts and dry-weight determinations of cell suspensions. The volumes are those shown in Table 1.
[c] Cells without visible slime capsule.
[d] ND, Not determined.

the hyphae even after homogenization.

The satellite bands in the density gradient only occurred with isolates which had loosely attached extracellular material. By microscopical examination of the major (low-density) and satellite (high-density) bands of these fungi, it was found that the satellite band contained hyphae which were almost free from extracellular material, whereas the major band contained hyphae with large amounts of extracellular material.

Table 3 also shows the percent dry weight of fungal material after suction on a membrane filter. The values obtained varied from 11% for *Mycena metata* to 30% for *Collybia butyracea*.

The last column in Table 3 shows the calculated percent dry weight based on biovolume measurements by fluorescence microscopy. These values varied from 21% for *Trichoderma viride*, which is free from extracellular materials, to 382% for *Mycena epipterygia*, with hyphae more or less embedded in loosely adhered material.

As shown in Table 4, neither fungal mycelia from spruce needle litter nor hyphae grown on milled sterile spruce needles contained the type of extracellular material found around hyphae grown in malt extract solution.

By cultivation of the fungi on sterile membrane filters placed on top of malt extract agar plates, extracellular material was not produced (Table 5). This hyphal material gave only one band in density gradient centrifugation, with a buoyant density of 1.10 to 1.11 g/cm^3. The cell diameter of the basidiomycetes varied between 2.6 and 3.2 μm.

TABLE 3. Diameter of fungal hyphae, percent dry weight of mycelial pads, buoyant density, and calculated percent dry matter based on measurements of biovolume of mycelia cultivated in liquid media

Fungi	Diam (μm)	Buoyant density (g/cm^3)		Avg buoyant density of main band (g/cm^3)	Presence of extracellular material[a]		% Dry wt (wt/wt) of mycelial pads after suction	Calculated % dry matter (wt/wt) from biovolume measurements
		Major band	Satellite band		Type 1	Type 2		
Mortierella sp.	5.1	1.036–1.072		1.054	–	–	22	24
Trichoderma viride	4.9	1.078–1.085		1.081	–	–	20	21
Trichoderma sp.	5.1	1.072–1.075		1.074	–	–	21	25
Clitocybe candicans	2.5	1.072–1.078		1.075	+	–	24	150
Collybia butyracea	2.3	1.072–1.082		1.077	+	–	30	143
Marasimus androsaceus	2.7	1.058–1.062		1.060	+	–	22	63
Micromphale perforans	2.6	1.058–1.062		1.060	+	–	21	57
Mycena epipterygia	2.0	1.028–1.038	1.095–1.110[b]	1.033	–	+	15	382
Mycena metata	4.4	1.016–1.038	1.100–1.110[b]	1.027	–	+	11	63
Mycena rosella	2.9	1.034–1.038	1.105–1.110[b]	1.036	–	+	14	35

[a] Type 1, extracellular material firmly adhered to the hyphae; type 2, extracellular material loosely attached to the hyphae.
[b] Faint band.

TABLE 4. Buoyant density and presence of slime adhered to fungal hyphae in either natural spruce forest or cultivated on sterile milled spruce needles

Source of fungal material	Buoyant density of free hyphal layer (g/cm³)	Presence of extracellular material
Spruce needle litter		
Location 1	1.08–1.10	–
Location 2	1.10–1.11	–
Location 3	1.09–1.13	
Pure cultures on sterile spruce needles		
Clavariadelphus ligula	1.08–1.11	–
Clitocybe candicans	1.07–1.09	–
Collybia butyracea	1.08–1.11	–
Mycena epipterygia	1.08–1.11	–
Mycena metata	1.08–1.13	–
Mycena rosella	1.09–1.11	–

The percent dry weight, calculated from the measurements of the biovolume and biomass (Table 5), varied from 18 to 22% for *M. epipterygia* and *C. butyracea*, respectively.

A significant correlation was found between the percent dry weight (weight/weight) of bacteria and their specific volumes, i.e., the inverse value of their buoyant densities. In Fig. 1A, the specific volume is plotted against the dry-matter content (weight/weight) together with the regression function as calculated with the percent dry weight as the independent variable. The dry-matter values used in the figure are those obtained from the investigations of the cell suspensions.

Similarly, a significant correlation was found between the dry-matter content of the hyphae after suction on the filter and their specific volumes. The data from Table 5 are plotted in Fig. 1B together with the regression function as calculated with the percent dry weight as the independent variable.

Assuming that the partial specific volume of the water in cells is 1 cm³/g, the following equation may be used to calculate the partial specific volume of the dry matter in the cells:

$$V_d = 1 - \frac{1 - V_w}{DW}$$

where V_w is the specific volume of the wet cell (inverse value of the buoyant density), V_d is the partial specific volume of the dry matter (cubic centimeters per gram), and DW is the decimal expression of the percent dry weight (weight/weight). Using values from the regression functions in Fig. 1, we obtained values for V_d between 0.69 cm³/g (DW = 0.15; V_w = 0.961) and 0.79 cm³/g (DW = 0.4; V_w = 0.92) for bacteria and 0.73 cm³/g for fungi.

DISCUSSION

The procedure of fixation and washing of the bacterial cells used in this work led to a slightly increased buoyant density of capsulated bacteria. This increase may be attributed to loss of extracellular slime with a lower buoyant density than that of the cell. The fixation and washing had no detectable influence on the buoyant density of noncapsulated cells, indicating that the initial volume and dry weight of the cells were not altered by this procedure.

The presence of extracellular material around fungal hyphae was dependent on the growth conditions. When grown in liquid culture, some of the basidiomycetes produced either loosely bound or a sheet of tightly bound extracellular material (Table 3). This material considerably affected the buoyant densities of the fungi, and an exact determination of the percent dry weight of cells plus extracellular material was precluded, since the volume of the latter could not be determined by microscopy due to the irregular shape.

Extracellular material was not produced when the fungi were grown on membrane filters on top of a solidified growth medium (Table 5). Mycelia of different species produced in this way showed similar buoyant density values of 1.10 to 1.11 g/cm³. Similar values were found for fungal mycelia released directly from forest needle litter and for hyphae of different fungal isolates grown in sterile needle litter (Table 4). Thus, mycelia grown on sterile filters seem to give relevant information as to the buoyant density and consequently the dry-matter content of fungal hyphae under natural conditions.

The suction of water from the bacterial pellets and fungal mats by applying an 81-kPa vacuum was not expected to remove water from the interior of the cells, since a water potential between 405 and 2,026 kPa is found in microbial cells (13). Some water necessarily remains between the cells after suction. Hence, the percent dry weight of the pellets obtained in this way represents a minimum estimate of that in the cells. This seems to be true for the microbial cells without capsules or other extracellular materials, as the percent dry weight of the pellets was practically identical to or lower than the values obtained by microscopical counting and dry-weight determination. Two capsulated bacteria behaved differently, the percent dry weight of the pellets after suction being substantially higher than that obtained by microscopical counting and dry-weight determination. This may be due either to loss of capsular material with low dry-matter content through the filter or, more probably, to loss of water from the capsular material during suction.

TABLE 5. Diameter of fungal hyphae, percent dry weight of mycelial mats, and calculated percent dry weight based on measurements of biovolume of mycelia cultivated on membrane filters

Fungi	Diam of cells (μm)	Buoyant density (g/cm³)	Avg buoyant density (g/cm³)	Bio-volume (μl/ml)	Dry matter (mg/ml)	Calculated % dry wt (wt/wt)	% Dry wt (wt/wt) in mycelial pads after suction	Presence of extracellular material
Collybia butyracea	2.8	1.100–1.110	1.105	1.42	0.35	22	19	–
Micromphale perforans	2.6	1.100–1.110	1.105	0.72	1.16	19	17	–
Mycena epipterygia	3.2	1.095–1.110	1.103	0.40	0.08	18	16	–
Mycena metata	3.1	1.100–1.110	1.105	1.72	0.40	21	19	–

The hyphal dry weight values of 150 and 382% (Table 3) indicate that cellular dry matter accounts for only 5 to 20% of the total dry matter produced by the fungi under those conditions. With fungi lacking extracellular material, there was a general correspondence between the percent dry weight of hyphae, as calculated from the microscopical examination and dry-weight determination, and those of the mycelial mats after suction.

The values of the buoyant density of different microbial cells obtained in this work by gradient centrifugation were lower than the values observed in density gradients of CsCl (1.3 g/cm³ [9] and 1.5 g/cm³ [17]). The low buoyant density of cells in gradients of colloidal silica compared with that in other gradient materials has been observed by Bowen et al. (4) (For references, see *Percoll, Methodology and Application*, Pharmacia Fine Chemicals, Uppsala Sweden.) In preliminary experiments, we observed that both fungi and bacteria would sediment through a CsCl solution with a density of 1.38 g/cm³. This did not happen immediately, however, but took place after a few minutes of contact between the CsCl solution and the cells. This indicates that the buoyant density of the cells was seriously affected by the CsCl. Such phenomena were not observed with colloidal silica, as repeated or prolonged centrifugation did not change the buoyant density of the cells.

Figure 1 shows that there is a positive correlation between the specific volume and the dry-matter content of the cells, both for fungi and bacteria. One should expect all the points in the figure to lie on a straight line crossing the vertical axis at 1 cm³/g, provided that the dry matter of the different species have the same average partial specific volume. The great spreading of the points may be attributed to differences in the average partial specific volume of the dry matter, but it is probably due mainly to experimental errors. The experimental errors may be great, especially for bacteria.

The values from the regression functions in Fig. 1 were used to calculate the average partial specific volume of the dry matter in bacteria and fungi. The extreme values for bacteria are 0.69 and 0.79 cm³/g, whereas the regression function for fungi gives nearly the same value at any point, i.e., 0.73 cm³/g, because the vertical axis is crossed very close to 1.0 cm³/g.

These values are in general agreement with reported values for the major cell components or similar polymers, that is, fibrous and globular proteins, 0.70 to 0.75 cm³/g (21); DNA and RNA, about 0.51 cm³/g (3); and polysaccharides (cellulose and starch), 0.60 to 0.80 cm³/g (24).

The relatively high buoyant densities of 1.3 (9) and 1.5 cm³/g (17) obtained in CsCl gradients may be attributed to dehydration of the cells by osmotic action (20) of the CsCl solution or to the possibility that CsCl penetrates the cell membrane. Our observation that the cells started to sediment after a few minutes of contact with the CsCl solution may support both explanations. This would imply that the observed buoyant density of cells in CsCl gradients is that of the cell components alone and not that of the intact cells.

Unfortunately, the densities observed in CsCl gradients have often been interpreted as the buoyant density of the intact cells (9, 17, 23). In fact, to fit a buoyant density of 1.3 g/cm³, one must assume a dry-matter content of 90% if the partial specific volume of the dry matter is 0.75 cm³/g. If an extreme value of 0.5 cm³/g is assumed for the partial specific volume, the dry-matter content must be 46% to fit the buoyant density of 1.3 g/cm³. Even more peculiar assumptions must be made to explain a density of 1.5 g/cm³ of the intact cell.

van Veen and Paul (23) have studied the amount of dry matter in bacteria, fungi, and yeasts grown under different water stress conditions. Their data on hyphae are in general agreement with our results, although their highest values (0.41 g [dry weight] per cm³ of cell volume) are somewhat higher than our observations. Their results with bacteria are more difficult to understand. In *Arthrobacter globiformis*, they observed up to 1.3 g (dry wt) per cm³ of cell volume, the highest value for *Enterobacter aerogenes* was 1.2 g/cm³, and the average of all observations was 0.8 g/cm³. We have shown in this study that the presence of extracellular

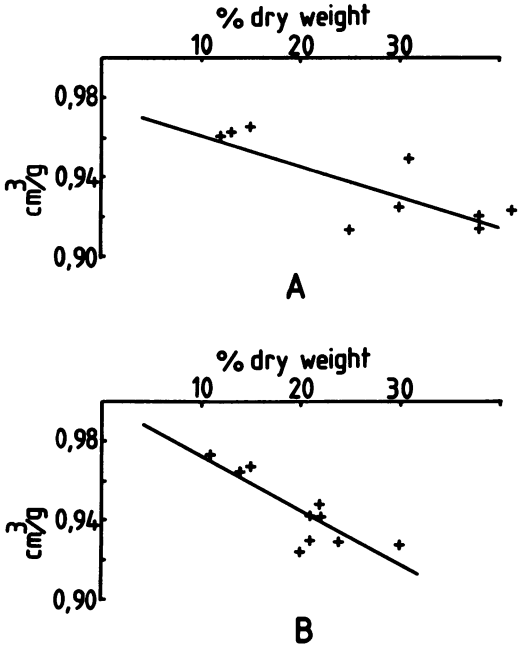

FIG. 1. Correlation between the percent dry weight and specific volume (cubic centimeters per gram) of bacterial cells (A) and fungal hyphae (B).

slime can lead to such anomalous values. van Veen and Paul (23) did not investigate whether their isolates had extracellular material, although one of the species studied, *E. aerogenes*, is known to contain capsulated strains (5). It is therefore tempting to speculate that their anomalously high values may be due to extracellular materials.

van Veen and Paul (23) have calculated an average of their observations at all levels of osmotic tension, that is, 0.8 g (dry matter) per cm^3 of cell volume, thus including clearly anomalous values, such as 1.3 g (dry matter) per cm^3 of cell volume. This average value has been used in later studies (18). A possible explanation for this very high average value is sought in speculations about bacterial response to high water stress conditions, although the value is obtained from equal numbers of observations at 0, 3.25, and 13.75 atmospheres of osmotic tension.

A large increase of the ash content due to water stress was also reported (23), which was taken to rationalize the data on the dry-matter content. One can assume that this increase was due to accumulation of KCl in the cells, since this has been shown to be used by some microorganisms as a compatible solute during water stress (19). However, 0.35 M KCl in the liquid would be sufficient to counteract the water stress of 13.75 atmospheres (6) which was applied to the cells, and this could hardly explain the very high dry-matter contents and ash contents reported (23). Accumulation of amino acids or polyols has also been shown to occur during water stress (19). Such an accumulation could possibly affect the dry-matter content more seriously than accumulation of KCl, due to a higher weight per molecule which contributes to the osmotic activity. The ash content, however, would be unaffected.

The similarity in buoyant density between the pure cultures and the cells released from soil (Table 2) necessarily indicates that the dry-matter contents are similar, provided that the major cell compounds occur in the same proportions in cells grown in shake cultures and in soil. The fact that 60% of the bacterial cells from the clay loam soil sedimented through the gradient was attributed to adsorption of clay minerals to these cells, rather than assuming a higher cell density. This is supported by the fact that with an organic soil containing very little clay (1 to 2%), 70% of the cells were recovered in the bacterial band of the Ludox gradient (L. Bakken, Ph.D. Thesis, Agricultural University of Norway, 1982), and with a well-drained peat soil, it was observed that 100% of the cells were recovered in the bacterial band (L. R. Bakken and R. A. Olsen, unpublished data).

To convert bacterial cell volume into biomass dry weight, we therefore propose that the average values of our results with noncapsulated bacterial isolates should be used: a buoyant density of 1.07 g/cm^3 and a dry-matter content of 30%. It must be clear, however, that the dry weight of the extracellular material is not included. We have shown in this study that the amounts of dry matter in the capsule are quite substantial compared to those in the cell. Capsules are widespread among soil bacteria (2, 7) and may contribute substantially to the estimated biomass if included. To convert the volume of the extracellular material into dry weight, we need some idea of its density and dry-matter content. This has been calculated from our results with capsulated bacteria by assuming that the cells within the capsule have a buoyant density of 1.07 g/cm^3 and a dry-matter content of 30%. The calculated values for capsular buoyant density are 1.084, 1.039, and 1.033 g/cm^3 and for capsular dry-matter content 44, 12, and 7% for isolates 1, 3, and 5, respectively.

An average of these results is of little value, since we evidently have two different types of capsules with widely different physical properties. At present, it is not possible to say which type of capsule dominates in soil, nor do we have a simple method for detecting slime capsules during direct microscopical counts of soil bacteria.

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LITERATURE CITED

1. **Babiuk, L. A., and E. A. Paul.** 1970. The use of fluorescein isothiocyanate in the determination of bacterial biomass of grassland soil. Can. J. Microbiol. **16**:57–62.
2. **Bae, H. C., and L. E. Casida, Jr.** 1973. Responses of indigenous microorganisms to soil incubation as viewed by transmission electron microscopy of cell thin sections. J. Bacteriol. **113**:1462–1473.
3. **Birnie, G. D., D. Rickwood, and A. Hell.** 1973. Buoyant densities and hydration of nucleic acids, proteins and nucleprotein complexes in metrizamide. Biochim. Biophys. Acta **331**:283–294.
4. **Bowen, R. A., J. M. St. Onge, J. B. Colten, Jr., and C. A. Price.** 1972. Density gradient centrifugation as an aid to sorting planctonic organisms. I. Gradient materials. Mar. Biol. **14**:242–247.
5. **Buchanan, R. E., and N. E. Gibbons.** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. **Campbell, G. S., and W. H. Gardner.** 1972. Water potentials in bars of KCl in solutions at temperatures between 0 and 40°C, p. 304. *In* R. W. Brown and B. P. van Haveren (ed.), Psychrometry in water relations research. Utah Agricultural Experiment Station, Utah State University.
7. **Casida, L. E., Jr.** 1971. Microorganisms in unamended soil as observed by various forms of microscopy and staining. Appl. Microbiol. **21**:1040–1045.
8. **Frankland, J. C., D. K. Kindley, and M. J. Swift.** 1978. A comparison of two methods for the estimation of mycelia biomass in leaf litter. Soil Biol. Biochem. **10**:323–333.
9. **Fægri, A., V. L. Torsvik, and J. Goksøyr.** 1977. Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. Soil Biol. Biochem. **9**:105–112.
10. **Harley, J. L.** 1971. Fungi in ecosystems. J. Ecol. **59**:653–678.
11. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
12. **Jenkinson, D. S.** 1976. The effect of biocidal treatments on metabolism in soil. IV. The decomposition of fumigated organisms in soil. Soil Biol. Biochem. **8**:203–208.
13. **Kilbertus, G., J. Proth, and B. Vervier.** 1979. Effect de la dessication sur les bactieres gram-negative d'un sol. Soil Biol. Biochem. **11**:109–114.
14. **Lindeberg, G., and M. Lindeberg.** 1964. The effect of pyridoxine and amino acids on the growth of *Marasmius perforans*. Fr. Arch. Microbiol. **49**:86–95.
15. **McLaren, A. D.** 1973. A need for counting microorganisms in soil mineral cycles. Environ. Lett. **5**:143–154.
16. **Modess, O.** 1941. Zur Kenntnis der Mycorrhizabildnen von Kiefer und Fichte. Symb. Bot. Ups. **5**:1–146.
17. **Parkinson, D., T. R. G. Gray, and S. T. Williams.** 1971. Methods for studying the ecology of soil microorganisms. International Biological Programme handbook no. 19. Blackwell Scientific Publishers, Oxford, England.
18. **Paul, E. A., and R. P. Voroney.** 1980. Nutrient and energy flows through soil microbial biomass, p. 215–237. *In* D. C. Ellwood, N. N. Hedger, M. J. Latham, J. M. Lynch, and J. H. Slater (ed.), Contemporary microbial ecology. Academic Press, Inc., London.
19. **Reid, D. S.** 1980. Water activity as the criterion of water availability, p. 15–27. *In* D. C. Ellwood, N. N. Hedger, M. J. Latham, J. M. Lynch, and J. H. Slater (ed), Contemporary microbial ecology. Academic Press, Inc., London.
20. **Ruffilli, D.** 1933. Untersuchungen über das spezifische Gewicht von Bakterien. Biochem. Z. **263**:63–74.
21. **Tanford, C.** 1961. Physical chemistry of macromolecules. John Wiley & Sons, London.
22. **Uhlen, G.** 1978. Nutrient leaching and surface runoff in field lysimetres on a cultivated soil. I. Runoff measurements, water composition and nutrient balances. Sci. Rep. Agric. Univ. Norway **57**:27.
23. **van Veen, J. A., and E. A. Paul.** 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions to biomass and their nutrient content. Appl. Environ. Microbiol. **37**:686–692
24. **Weast, R. C.** (ed.). 1977. Handbook of chemistry and physics, 56th ed. Chemical Rubber Co., Cleveland.
25. **Wolff, D. S.** 1975. The separation of cells and subcellular particles by colloidal silica density gradient centrifugation. Methods Cell Biol. **10**:85–104.