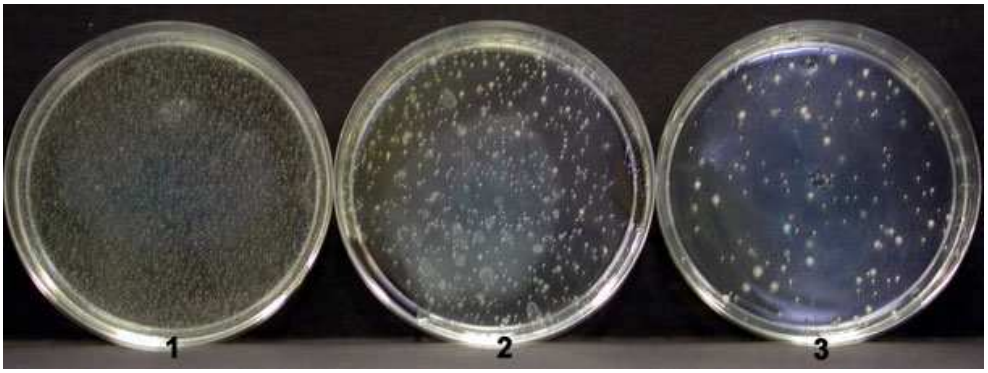
HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY

3. BIOMASS CALCULATION



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3. BIOMASS CALCULATION

When we use the term biomass we refer to the amount of mass of living material expressed as grams or calories (joules) per ml or g of sample and, it measures the amount of energy stored in a given segment of a biological community. There are different methods to quantify the biomass of a population/microbial community. Some of these methods are very simple and do not require of complex manipulations, other methods are more complex and indirect measures are needed. We will see some simple cases.

One direct and simple method for quantifying the biomass of a population is the estimation of the **dry weight per g or ml of sample** (Figure 1). It requires a weighing scale (accuracy g x 0.001), a recipient for placing the sample which can be made with an aluminum foil, and an oven to dry the sample. This method is valid, and widely used, with pure cultures (yeasts, etc.). But its use with natural samples (sediments, seawater samples, etc.) can be problematic.

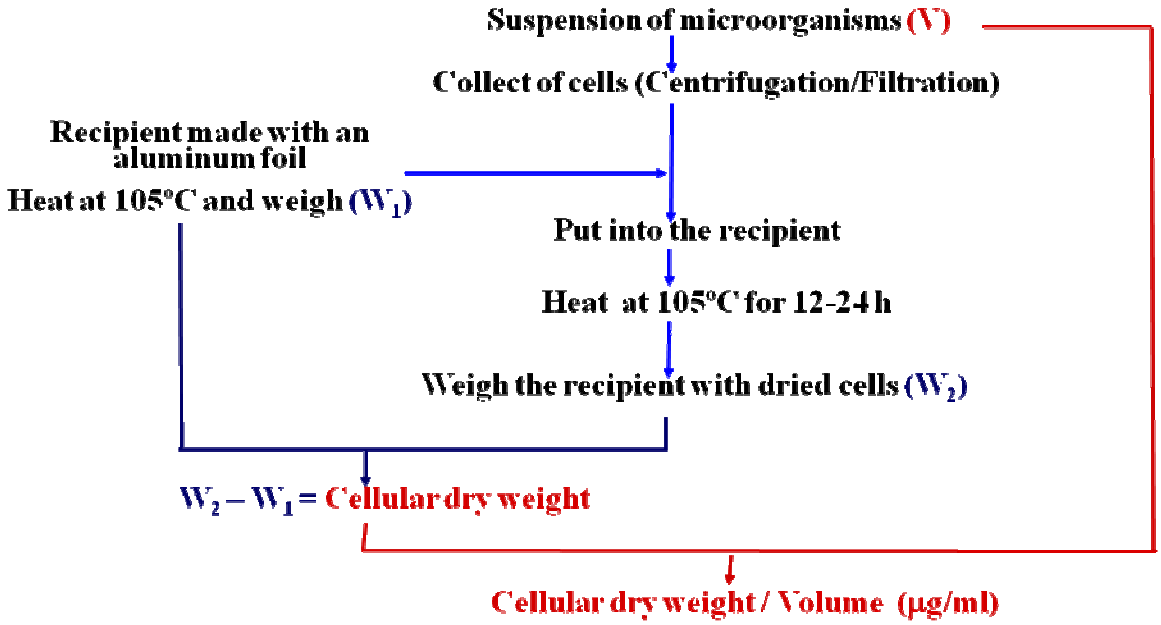

Figure 1. Scheme for the determination of dry weight

Again, a solution to the following problems must be proposed.

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3.1. To determine the dry weight of a culture of *Saccharomyces cerevisiae*, 10 ml of a yeast suspension was centrifuged at 5,000 g. After removing the supernatant, 3 ml of sterile saline solution were added and stirred until homogenization. 1 ml of this new suspension was placed into a metal recipient (weight of the empty recipient = 0.300 g) and dried in oven at 105°C for 24 h. After this drying period, the weight of the recipient was 0.350 g. What is the dry weight of the culture expressed as g/ml?

Another valid method for determining the biomass of natural populations, more complex and laborious, is **the determination of biomass through biovolume** (Figure 2). In this method, measures of the length, width or diameter of a certain number of microorganisms by sample are recorded in preparations for photonic or epifluorescence microscopy.

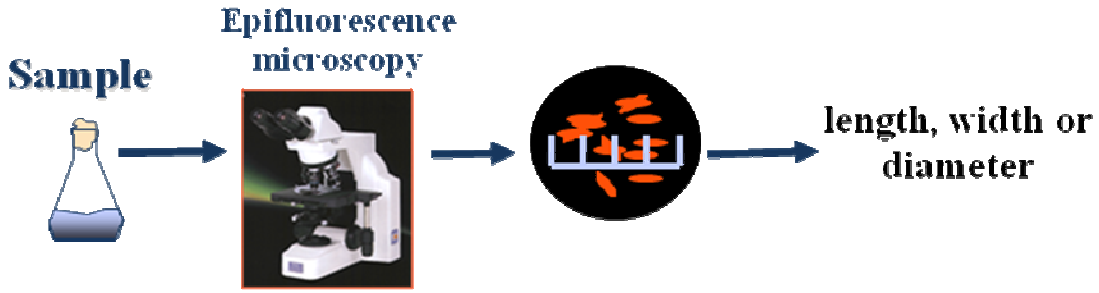

Figure 2. Scheme for measurement of bacterial size

Microorganisms are assigned to more or less complex geometric forms, in order to obtain an equation which allows calculating the biovolume (Figure 3).

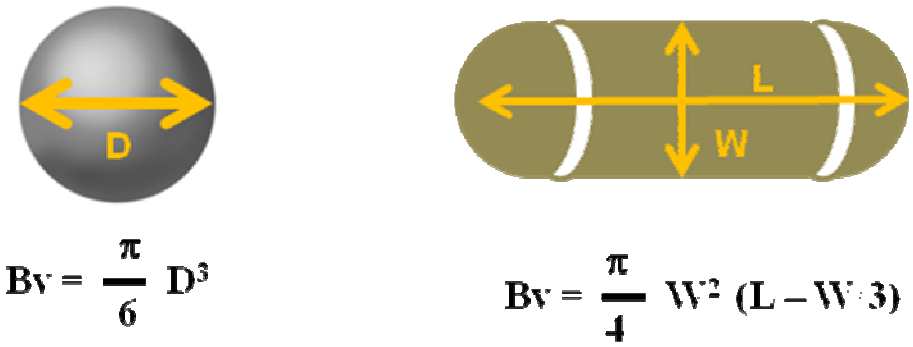

Figure 3. Geometric form and equation defining morphology and biovolume of coccus and bacillus.

The following equation permits to obtain the biomass of population:

Biomass (μg C/ml) = N (No. cells/ml) x Biovolume (μm^3/cell) x F (μg C/m^3)

Where: N = number of organisms per ml of sample examined,

Bv = biovolume obtained as described above,

F = conversion factor (quantity of carbon by cellular volume). In the literature there are different values of F calculated as pure cultures, both for natural samples and for primarily water systems.

3.2. A bacterial sample was stained with acridine orange and prepared for epifluorescence microscopy. Using an image analysis system we proceeded to measure the length and width of 10 cells, obtaining the following results:

Cell	W: width (μm)	L: Lenght (μm)
1	0.51	1.0234
2	0.50	1.0342
3	0.51	1.1091
4	0.50	1.0921
5	0.49	1.0456
6	0.49	1.1001
7	0.50	1.1000
8	0.50	1.0761
9	0.51	1.0345
10	0.49	1.0555

If the density of the culture is 4.8 10^7 bacteria/ml and F (conversion factor) has been estimated in 164 fg C/μm^3, which is the biomass of the culture?

SOLUTIONS

3.1. Dry weight (g/ml) for the *Saccharomyces cerevisiae* culture?

10 ml suspension/ 3 ml diluent = 3.33 = Concentration Factor
0.350 – 0.300 = 0.050 g

$$\frac{0.050\ g}{3.33\ ml} = \boxed{0.015\ g/ml}$$

3.2. Biomass of the culture?

Cell	A: Width (µm)	L: Lenght (µm)	Biovolume (µm³)
1	0.51	1.0234	0.1743
2	0.50	1.0342	0.1767
3	0.51	1.1091	0.1918
4	0.50	1.0921	0.1817
5	0.49	1.0456	0.1664
6	0.49	1.1001	0.1767
7	0.50	1.1000	0.1833
8	0.50	1.0761	0.1786
9	0.51	1.0345	0.1766
10	0.49	1.0555	0.1682
Average biovolume (µm³/bacterium)			0.1717

Biomass (µg C/ml) = N x Bv x F

Biomass (µg C/ml) = 4.8 10^7 bacteria/ml * 0.1717 µm³/bacteria * 164 fg C/µm³ =

1.3916 fg C/ml