MICROSCOPIC YEAST CELL COUNTING

This procedure provides a method for determining yeast cell concentration using a hemocytometer and a microscope. The procedure relies on the enumeration of cells within a specific number of microscopic fields to determine the concentration of yeast in a population.

Reagent

(a) *Diluent*, 0.5% sulfuric acid or 0.5*M* disodium ethylenediaminetetraactetic acid (EDTA) solution adjusted to pH 7.0 with NaOH. Other solutions capable of deflocculating yeast may be substituted.

Apparatus

(a) *Microscope*, approximately 400–500× magnification, with bright-field illumination.
(b) *Hemocytometer*, open type with improved Neubauer ruling and with hemocytometer cover slip.
(c) *Pipets*, 1.0-mL fine tip, 10-mL volumetric.
(d) *Counting device*, such as hand tally counter or equivalent.
(e) *Magnetic stirrer.*
(f) *Magnetic stir bar*.
(g) *Volumetric flask*, 100-mL.
(h) *Beaker*, 250-mL.

The hemocytometer used for cell determinations consists of a one-piece thermal and shock-resistant glass chamber with two counting areas of nine ruled blocks each separated on three sides by a moat. The ruled area used for yeast cell counting is the central block of the nine ruled blocks, composed of 25 squares, each of which contains 16 smaller squares. This entire ruled central area measures 1 mm on each side, with a total area of 1 mm^2. Each of the 25 squares measures 0.2 mm, with an area of 0.04 mm^2. The depth of the counting area is determined by the cover glass supports, which are usually 0.1 mm above the counting area. The volume of liquid directly over the ruled area can be calculated using the formula:

$$\text{Volume } (V) = \text{length } (L) \times \text{width } (W) \times \text{height } (H)$$

If $L = 1$ mm, $W = 1$ mm, and $H = 0.1$ mm, then the volume becomes 1 mm × 1 mm × 0.1 mm or 0.1 mm^3 or, converting to centimeters, 0.001 cm^3 = 1×10^{-3} cm^3.

Because other types of counting chambers are available, it is imperative that the exact dimensions of the chamber in use be known. For such, consult the directions accompanying the chamber.

Method

Cleaning the counting chamber

The counting chamber must be clean and dry before use. Dirty counting chambers may influence the volume of sample over the counting area and therefore lead to erroneous results.

Clean the counting chambers and cover slip with tap water. It may be necessary to scrub the ruled area. Scrubbing should be done in all directions if necessary. Dry the chamber and cover slip using silk or other lintless material.

Positioning the cover slip

The cover slip should be centered over the counting area such that both counting sections are equally covered and equal amounts of cover slip project over the cover glass slide supports. The cover slip should be placed by gently sliding the slip across the surface of the counting chamber such that Newton's rings become visible.

Preparing the yeast sample

The sample to be counted must be well mixed, degassed, and diluted, if necessary. If the sample to be counted does not require dilution, degas and then mix a 100-mL sample for a minimum of 5 min using a magnetic stirrer. If dilution is necessary, apparatus such as volumetric pipettes or air-displacement pipettes (Gilson pipettes or equivalent) should be used to perform the dilution. To deflocculate cells, 0.5% sulfuric acid or EDTA (0.5*M*, pH 7.0) may be used as a diluent (reagent a). The final dilution should ideally provide a 100-mL sample, which is then mixed for a minimum of 5 min using the magnetic stirrer before filling the hemocytometer. If smaller samples are required, particular care should be taken to ensure accuracy when diluting and preparing them. It is not recommended to handle brewery yeast samples of less than 1 mL.

Filling the hemocytometer

Because the counting area and the correctly positioned cover glass determine the volume over the counting area, it is extremely important to fill the hemocytometer correctly. The sample is constantly stirred during the replicate counting period. A portion of the sample is taken up in a fine-tip pipet, after which the tip of the pipet is wiped dry. After expelling three or four drops of solution, allow a small drop of sample to flow between the hemocytometer and cover slip of both sides of the hemocytometer. The entire counting area must be filled completely, but no part of the sample should extend into the moat. Let the prepared slide stand for a few minutes to allow yeast to settle.

Counting

Counting the yeast cells within the 1-mm^2 ruled area will be accomplished by counting all of the cells in the entire ruled area, 25 squares. A total of 10 counting areas

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should be counted and averaged to ensure statistical validity.

To eliminate the possibility of counting some yeast cells twice, it is necessary to standardize the counting technique. Cells touching or resting on the top and right boundary lines are not counted. Cells touching or resting on the bottom or left boundary lines are counted. Yeast cells that are budded are counted as one cell if the bud is less than one-half the size of the mother cell. If the bud is equal to or greater than one-half the size of the mother cell, both cells are counted. To obtain an accurate yeast cell count, it is advisable to count no fewer than 75 cells on the entire 1-mm^2 ruled area and no more than about 48 cells in one of the 25 squares. Counts from both sides of the slide should agree within 10%. If a dilution is used, the dilution factor must be used in the calculation. Data should be reported to two significant figures.

Calculation

Number of cells/mL
= total cells in central 25-square ruled area
$\times$ dilution factor (if any) $\times (1 \times 10^4)$

Example

A fermenter sample collected 48 h after pitching was submitted for yeast cell count. The actively fermenting sample was degassed by vigorous shaking, then diluted 1:10 with 0.5% deflocculent (reagent a), mixed 5 min on a magnetic stirrer, and loaded onto the hemocytometer. After approximately 3 min, 600 cells were counted in the 25 squares of one side of the hemocytometer.

Yeast cells/mL
$= 600 \times$ dilution factor $\times 10^4$
$= 600 \times 10 \times 10^4$
$= 60 \times 10^7$ cells/mL

Precision

A collaborative study of samples having grand means of 438.6 and 393.4×10^5 cells/mL provided coefficients of variation (cv) of 9.1 and 9.8, respectively (1).

Reference

1. American Society of Brewing Chemists. Report of Subcommittee on Improved Microscopic Yeast Cell Counting. *Journal* 46:123, 1988.

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