Counting Yeast

Using a Hemacytometer

One of the most important controls in fermentation is the population of yeast at the beginning of fermentation (known as pitching rate). The simplest and cheapest means of accurately determining the pitching rate is the use of a hemacytometer and a microscope. A hemacytometer is an specialized slide which has a counting chamber with a known volume. By placing a diluted slurry of pitching yeast on the slide, the amount of cells in the chamber can be counted, giving a concentration of cells/ml in the pitching yeast.

It is also possible to estimate the viability of the pitching yeast, by staining the slurry with methylene blue before counting. This stain is metabolized by viable cells, and hence they will not stain blue, while dead cells will take up the stain and turn blue. Unfortunately this method is only accurate for viabilities above 85-90%, and results indicating a viability below 85% should be considered relatively meaningless. In fact recent research indicates that viabilities below 95% may not be accurate.

To use a hemacytometer you will need a microscope with a 40× objective, some pipettes (including graduated pipettes for accurate measurement), glassware for dilutions, methylene blue stain, and a counting device.

The first step in measuring the concentration of your pitching yeast is to get an representative sample. Make sure that the yeast is homogenous by vigorously mixing before sampling, and then carefully pipette 1 ml of the yeast into a 100 ml volumetric flask. By filling this flask with 99 ml of water, you will have a 100:1 dilution. It is also possible to do serial dilutions to achieve the same result (two 10:1 dilutions), and you may need to do more dilutions depending on the concentration of your sample. After your dilution is finished, place a drop of methylene blue into the sample, and allow it to stand for a few minutes.

Place the clean, dry hemacytometer on the stage of the microscope. Place the cover slip carefully on top of the counting chamber, making sure that both sides are resting on the raised area. Draw a homogenous sample of the diluted yeast slurry into a Pasteur pipette and place the tip on the filling notch of the hemacytometer. Allow the counting chamber to fill, avoiding spill over into the overflow area. If the slurry overflows onto the raised area it will raise up the cover glass, changing the volume of the counting chamber and making the count inaccurate.

Focus the microscope at 400× and count five of the areas inside the grid (see article for which to count). First count the total number of cells, then the total number of dead cells for each area. Note your count on a grid laid out like the counting chamber, to allow for easier interpretation.

THE MATH

Now that you have a count of the number of cells in the chamber you must determine the concentration of cells in the slurry and then the amount of pitching yeast you will require. First you must know the volume of the counting chamber, which has dimensions of 1 mm × 1 mm × 0.1 mm. Since the dimensions of 1 ml (also known as a cubic centimeter) are 1 cm × 1 cm × 1 cm, (or 10 mm × 10 mm × 10 mm), you can determine the volume of the chamber with some fairly simple math. Change the dimensions into centimeters and multiply them, and you will have the volume in cubic centimeters (cm^3) or mls.

$$0.1\ cm \times 0.1\ cm \times 0.01\ cm = 0.0001\ cm^3 \text{ or } 1 \times 10^{-4}\ ml$$

Determine your viability by subtracting the dead cells from the total cells and dividing by the total cells.

$$\frac{\text{(Total cells) - (Dead Cells)}}{\text{Total Cells}} \times 100 = \text{Viability}$$

To get your viable count, subtract the dead cells from the total cells in the five counting areas.

$$\text{Total Cells - Dead Cells = Viable Cell Count}$$

Now take your Viable Cell Count of the five areas, and multiply by five to give an average count for the entire chamber. Divide by the volume of the chamber and multiply by your dilution factor to give the total cells per ml.

$$\frac{\text{(Viable Cell Count)(5)(dilution)}}{\text{(chamber volume)}} = \text{yeast cells/ml}_{\text{slurry}}$$

Counting Yeast

Next you need to determine the amount of yeast you will pitch into your fermentation. For most yeast, a healthy fermentation is achieved when the population of viable yeast is 1×10^6 cells per °Plato of the wort. This accounts for the requirement for increased population in higher gravity worts. So by multiplying the gravity by 1×10^6 you can determine your proper pitching rate per ml of wort.

$$\text{°Plato Wort} \times \frac{1 \times 10^6 \text{ viable cells/ml}_{wort}}{1 \text{ °Plato}} = \text{viable cells/ml}_{wort} \text{ (Pitching Rate)}$$

Next convert your volume of wort (probably in barrels) to ml and multiply by your pitching rate to get the total cells needed.

$$\text{bbl}_{wort} \times \frac{117.35 \text{ L}_{wort}}{1 \text{ bbl}_{wort}} \times \frac{1000 \text{ ml}_{wort}}{1 \text{ L}_{wort}} \times \text{viable cells/ml}_{wort} = \text{Total Cells Needed}$$

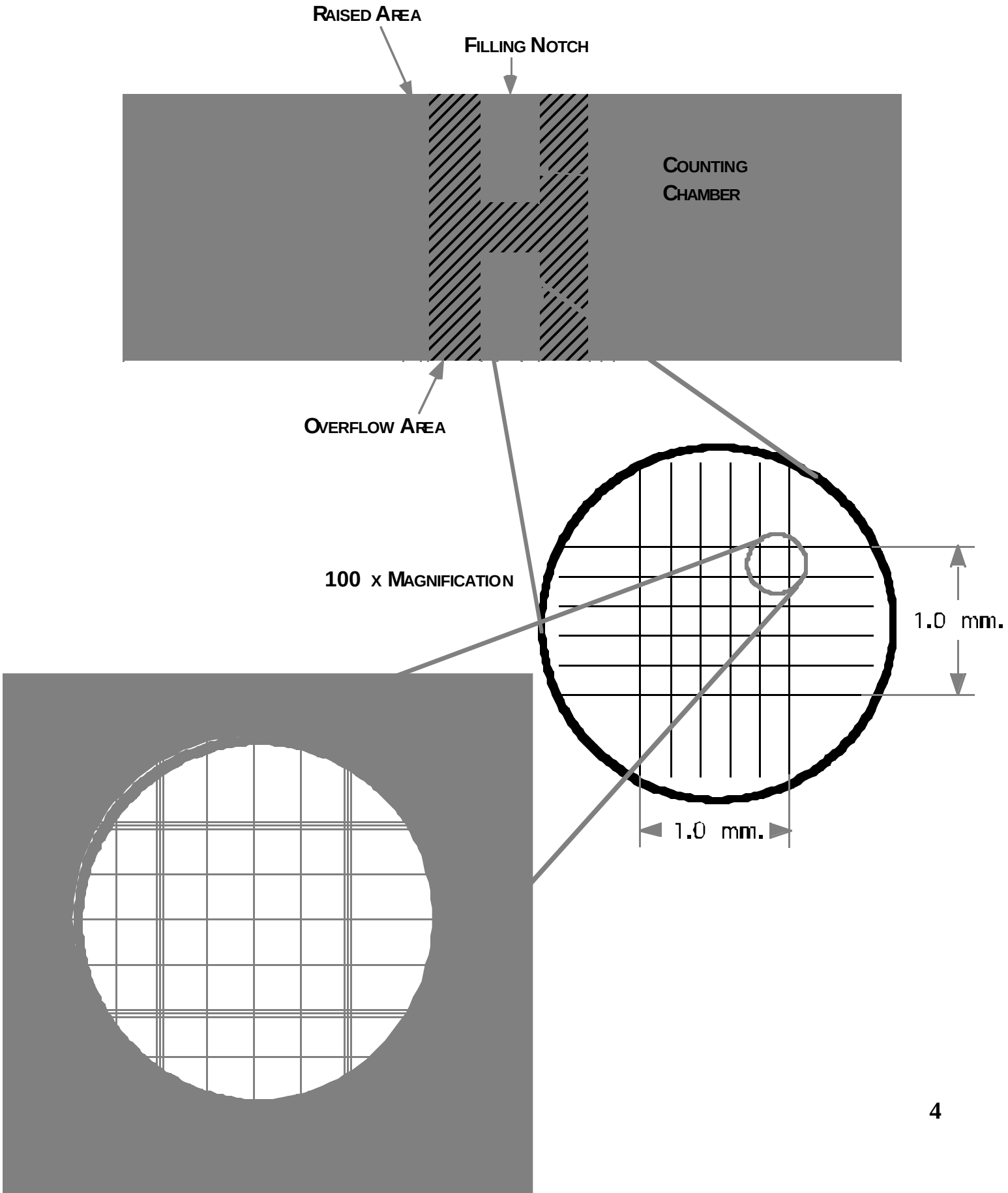
Finally, divide your total cells needed by the viable cells/ml to get your total pitching quantity.

$$\frac{\text{Total Cells Needed}}{\text{yeast cells/ml}_{slurry}} = \text{Volume of yeast slurry required (ml}_{slurry})$$

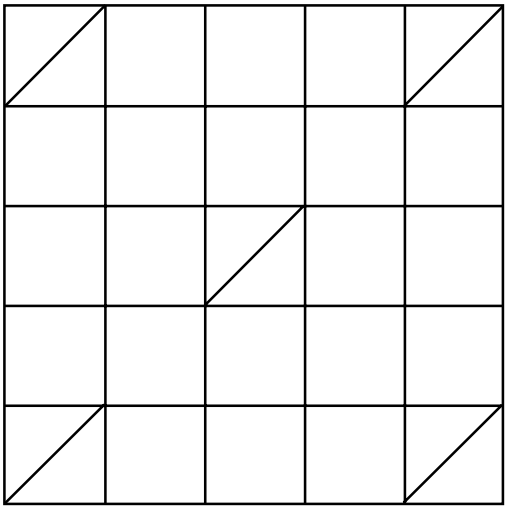
You can also convert this to gallons for easier use.

$$\text{ml}_{slurry} \times \frac{1 \text{ gal}_{slurry}}{3785 \text{ ml}_{slurry}} = \text{gal}_{slurry}$$

COUNTING YEAST



YEAST COUNT DATA SHEET



Dead Cell Count _____

Total Cell Count _____

Viable Cell Count _____

Viability % _____

Viable Cells/ml_{slurry} _____

Pitching Rate _____

Total Cells Needed _____

Volume yeast (ml_{slurry}) _____

gal_{slurry} _____