LAB 4: DAY 1 DIRECTIONS

PART I. INTRODUCTION
1. Listen and take notes on the background information about fermentation presented in class. Be able to address the following questions in the introduction of your lab report (you will need outside materials to answer some of these questions)

   a. What is fermentation? How is sugar converted to alcohol and CO₂?
   b. When making alcoholic beverages, what organisms are commonly used? What organism are we using?
   c. How is fermentation different from cellular respiration?
   d. Why is it important to keep organisms in anaerobic (without oxygen) conditions?
   e. Do yeast undergoing fermentation divide?
   f. What is our biological question?

   g. What is your hypothesis regarding?
      The amount of alcohol produced compared to the amount of sugar added?
      The change in the number of cells compared to 1) the amount of sugar and 2) during fermentation?
      The amount of CO₂ produced during the first week of fermentation?

PART II: LAB INSTRUCTIONS. At the end of lab, 1 group member will need to give me the complete data for their group

1. Form 5 groups, with 4 -5 members each. Write name of group members in lab notebook and on data worksheet. Inform the instructor at the end of the lab.

2. This is experiment will be a classroom effort. Therefore, each group will be making 1 sample that we are testing. I will distribute the complete data of everyone to the class through Moodle. Have 1 group member randomly draw the sample they will be making from the instructor.

   SAMPLE________________________M
SOLUTION CALCULATIONS AND INITIAL MEASUREMENTS

3. Calculate the mass of sugar you will need to add to 600mL to make the Molar solution of your sample. Put this calculation in your lab notebook. Report this amount the instructor for your group. Make your solution of sugar and grape juice using proper measuring techniques (what piece of equipment will you use to measure volume?)

4. Once you have made your sugar/grape juice solution, take initial measurements on the % alcohol content of both distilled water and your sugar/grape juice solution using the vinometer. The questions in italics should be answered in your lab report.
   • You will need 500mL to complete the experiment. Use the 100mL extra to take your measurements. DO NOT GO OVER 100mL for measurements.
   • Read the directions in the handouts about how to take measurements using the vinometer.
   • Measure and record the alcohol content of pure water. (What is the purpose of this measurement?)
   • Measure and record the alcohol content of your grape juice + sugar mixture. (What is the purpose of this measurement? How is it different from the measurement of pure water?)

INITIAL CELL NUMBER MEASUREMENTS

5. Next you will take measurements on the amount of yeast you are adding to the wine culture. Be sure to pay attention to all instructions regarding how to count cells… this is very likely a lab practical question.
   • Read about using the hemocytometer in the lab handouts and become familiar with the counting grid and its measurements
   • Focus onto the grid using a 40X objective lens
   • Pipette 10µL of well-mixed starter culture onto each loading chamber of the hemocytometer. Liquid/cell mixture should spread across the grid.
   • Count the number of cells in a 1mm² square of each grid. If you are unsure of what to count, ask the instructor.
   • Have each member of the group count the number of cells on each side of the chamber and record the results.
   • Using the formula in the handout, determine the number of cells / mL in the starter culture. This will tell you the concentration of cells that we are beginning with in the starter culture.
   • For your lab report: Preparation of starter culture: Starter culture was prepared by incubating ~0.5g of active dry baker’s yeast (Fleischmann’s All Natural Active Dry Yeast) to 50mL conical tube at room temperature overnight with the lid loosely capped.

6. Calculate the TOTAL number of cells that we are adding the grape juice/sugar solution.

7. Calculate the TOTAL number of cells present in the 500mL grape juice/sugar solution.

8. Once you have 1) made your sugar/grape juice solution and completed calculations; 2) had each group member count the number of yeast cells in the starting amount, inform the instructor and together, we will set-up the air-lock system in the back of the lab.
a. Draw the fermentation set-up in your lab notebook and use labels to describe what you see
b. What is the importance putting a piece of the set-up tubing in a flask of water?
c. Why is there an escaper hose?

INITIAL CO$_2$ or GAS MEASUREMENTS

9. Describe in your notebook the whether there are bubbles or gas forming
   1) AT the time of sample inoculation (when we added the yeast)
   2) 2 hours after inoculation (view videos posted on MOODLE)

You are free to leave when…

1) Your calculations are complete and your air-locked sample is prepared.
2) Our initial measurements from ALL lab group members are complete
3) Group data has been given to instructor.

LOOK ON MOODLE FOR 1) CLASS DATA SET and 2) VIDEO/PHOTOGRAPH DATA!!!!!!
Part I: Day 1
Date: ________________________ Sample ______________________

1. Vinometer measurements
A. What is the % alcohol in the water? Measure 2X - take average

B. What is the % alcohol for the wine + sugar? Measure 2X - take average

C. Was the measurement for the water compared to (wine + sugar) measurement similar or different?
   Explain why.

2. Measurement of the number of yeast cells
A. The number of cells in the starter culture

<table>
<thead>
<tr>
<th>Counts in each Field of View</th>
<th>Average Counts for Group Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Squares Counted</td>
<td>Total Cell Count</td>
</tr>
<tr>
<td>GRID 1</td>
<td></td>
</tr>
<tr>
<td>GRID 2</td>
<td></td>
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<tr>
<td>Average</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
</tr>
</tbody>
</table>

Calculate the amount of cells/mL of starter culture
   Use Average of counts for Group Members

B. The amount of cells added to the wine
   \[(\text{number of cells} / \text{mL}) \times 2 \text{ mL} = \text{Total number of cells added to culture}\]

C. Calculated number of cells / mL in the wine + sugar mixtures
   \[\text{cells/mL in culture} = \frac{(\text{total number of cells added to culture})}{500 \text{ mL}}\]

3. CO₂ Formation
A. Examine your solution at day 0, time 0. Is there formation of CO₂?
How to Measure the Alcohol Content of Wine

By Jim Walrod, eHow Contributor

The fermentation is over and you are ready to bottle the wine that you have been carefully nurturing. The taste is right but you cannot tell the alcohol content from taste. For that you need a device called a vinometer, an inexpensive tool found at brewing stores or online. Does this Spark an idea?

Other People Are Reading

Instructions

1. Withdraw a small sample from the fermentor. To get an accurate reading, especially with sweet wines, mix 1 teaspoon of the wine you are testing with 1 teaspoon of water in a clean container and stir the mixture well. Fill the reservoir of the vinometer with the prepared sample. Continue to fill the reservoir until some wine exits from the other end of the device. Make sure there are no air bubbles in the tube. If they appear tap lightly with your finger until the bubbles disperse.

2. Holding the vinometer upright, rotate it 180 degrees and carefully set it on a flat plate. Watch as the mixture drains and then record the reading when the sample is stabilized in the capillary tube.

Things You'll Need

- Vinometer

Sponsored Links

Density Measurement

Accurately Measure Mass Flow and Density with 2-Wire Coriolis Meters
EmersonProcess.com/MicroMotion

Related Ads

- Wine for Sale Online
- Red Wine
- Making Wine
- Homemade Wine
- Blood Alcohol Content

Bite Into Autumn: Just Add Pumpkin

alcohol content figure. You should check the alcohol content of your wine right up to the final bottling.

5 Another way to test for alcohol content is with a winemaker’s hydrometer. It requires a larger sample of the wine to be drawn. Since alcohol content needs to be monitored regularly, a hydrometer may not be appropriate for the small batch home winemaker.

Tips & Warnings

Clean the vinometer thoroughly after each use.

The vinometer is made of glass and is extremely fragile.

Related Searches

Vinometer
White Wine Glass
Wine Tasting Trips
Good Wine
Alcohol Hydrometer

More to Explore

How to Test for Alcohol Content in Homemade Wine
A Dangerous Duo: Alcohol and Depression (video) (Health Guru)
How to Make Wine High in Alcohol Content
Video Courses: Learn Shake (Lynda.com)

References

Vinometer

A vinometer is a piece apparatus that consist of an open, graduated glass tube with a small filling reservoir.

The vinometer's reservoir or funnel is filled with a small amount of wine being tested until some wine exits out at the other end. It is then turned around and placed on a flat surface, filling reservoir side down, and allowed to self drain.

The alcohol concentration level is read of the vinometer's scale, on the capillary, at the top of the liquid being measured, in %v/v alcohol.

The level of the liquid is determined by the modifying affect that alcohol has on the interfacial tension between water and glass and the opposing surface tension of water. The greater the alcohol concentration the less marked the liquids capillary action and the lower it will sit in the tube of the vinometer.

The vinometer is calculated on the basis of pure alcohol and water solutions, whereas wort is not a pure water/alcohol solution and the accuracy of a vinometer can not be relied upon.

Sugar interferes with the interfacial effects that a vinometer's action relies on and hence the method can only be applied to fully fermented wort. Similarly other components in wort can also interfere with accurate measurements.

As a rough approximation of the wort alcohol content, the use of a vinometer with an internal triangular cross section has been reported to give improved accuracy.

Basic rules you have to keep in mind

- Don't spend a fortune on these things. They should not cost more than $5 - $10.

Where to get it

- Check your local homebrew shop. They will have them - no doubt about it!
- Check the web if there is no homebrew shop in your area.
The Bright-Line Hemacytometer is molded from a single piece of thermal and shock-resistant glass. An H-shaped moat forms two counting areas, or plateaus. A "V" slash at the loading side of each plateau facilitates charting and reduces the possibility of overflow into the moat. Each plateau features enhanced Neubauer rulings. The ruled surface is 0.1mm below the cover glass, limiting the volume of blood or fluid over a square mm at 0.1cu.mm and over each of 400 squares (within the central square mm) to 0.00025 cu. mm. Contact of the flat, polished cover glass surfaces with cover glass supports produces an exact volume of fluid over the counting area. The difference in surface tension characteristics between the metallic surface on the chamber and the polished cover glass assures smooth capillarity for precise loading and more even cell distribution.

### Directions for Use

**Bright-Line / Dark-Line Counting Chambers** Catalog Numbers: 3100, 3110, 3200, 3500, 1490, 1492, 1475, & 1483

**Usage:** Cell Counts

- **Cell Depth:** 0.100mm +/- 2% (1/10mm)
- **Volume:** 0.1 Microliter
- **Ruling Pattern:** Improved Neubauer, 1/400 Square mm

Rulings cover 9 square millimeters. Boundary lines of the Neubauer ruling are the center lines of the groups of three. (These are indicated in the illustration below.) The central square millimeter is ruled into 25 groups of 16 small squares, each group separated by triple lines, the middle one of which is the boundary. The ruled surface is 0.10mm below the cover glass, so that the volume over each of the 16 small squares is 0.00025 cubic mm.
The number of cells per cubic millimeter = 
Number of cells counted per square millimeter × dilution (if used) × 10

The number of cells per milliliter = 
Number of cells counted per square millimeter × dilution (if used) × 10,000

One (1) Milliliter = 1000 cubic millimeters (cu mm)
One (1) Microliter (ul) = One (1) cubic millimeter (cu mm)

To clean the counting chamber: After completing the count, remove the cover glass and clean the counting chamber with water or a mild cleaning solution (10% solution of bleach). Dry the counting chamber with a soft cloth or wipe, or rinse with acetone.
Hemocytometer

From Wikipedia, the free encyclopedia

The hemocytometer or haemocytometer is a device originally designed for the counting of blood cells. It is now also used to count other types of cells as well as other microscopic particles.

The hemocytometer was invented by Louis-Charles Malassez and consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall.

Contents

- 1 Principles
- 2 Usage
- 3 Usage Tips
- 4 References

Principles

The ruled area of the hemocytometer consists of several, large, 1 x 1 mm (1 mm²) squares. These are subdivided in 3 ways; 0.25 x 0.25 mm (0.0625 mm²), 0.25 x 0.20 mm (0.05 mm²) and 0.20 x 0.20 mm (0.04 mm²). The central, 0.20 x 0.20 mm marked, 1 x 1 mm square is further subdivided into 0.05 x 0.05 mm (0.0025 mm²) squares. The raised edges of the hemocytometer hold the coverslip 0.1 mm off the marked grid. This gives each square a defined volume.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Area</th>
<th>Volume at 0.1 mm depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 1 mm</td>
<td>1 mm²</td>
<td>100 nl</td>
</tr>
<tr>
<td>0.25 x 0.25 mm</td>
<td>0.0625 mm²</td>
<td>6.25 nl</td>
</tr>
<tr>
<td>0.25 x 0.20 mm</td>
<td>0.05 mm²</td>
<td>5 nl</td>
</tr>
<tr>
<td>0.20 x 0.20 mm</td>
<td>0.04 mm²</td>
<td>4 nl</td>
</tr>
<tr>
<td>0.05 x 0.05 mm</td>
<td>0.0025 mm²</td>
<td>0.25 nl</td>
</tr>
</tbody>
</table>

The cell-sized structures to be counted are those which lie between the middle of the three lines on the top and right of the square and the inner of the three lines on the bottom and left of the square.

In an improved Neubauer hemocytometer (common medium), the total number of cells per ml can be discovered by simply multiplying the total number of cells found in the hemocytometer grid (area equal to the red square in picture on right) by $10^4$ (10000).

Usage

When a liquid sample containing immobilized cells is placed on the chamber, it is covered with a cover glass, and capillary action completely fills the chamber with the sample. Looking at the chamber through a microscope, the number of cells in the chamber can be determined by counting. Different kinds of cells can be counted separately as long as they are visually distinguishable. The number of cells in the chamber is used to
calculate the concentration or density of the cells in the mixture from which the sample was taken: it is the number of cells in the chamber divided by the chamber's volume (the chamber's volume is known from the start), taking account of any dilutions and counting shortcuts:

In the most common design, the volume of each large square is 0.1 mm$^3$. The cells in four large squares are counted and cells over or touching the lines on top and on the left are counted, but cells over or touching the right or bottom lines are ignored. The concentration in cells per ml = cells in four squares/4 $\times$ 10,000.[1]

Hemocytometers are often used to count blood corpuscles, organelles within cells, blood cells in cerebrospinal fluid after performing a lumbar puncture, or other cell types in suspension. Using a hemocytometer to count bacteria results in a 'total count' as it is difficult to distinguish between living and dead organisms unless Trypan blue is used to stain the non-viable cells.

Usage Tips

- Mix the original mixture thoroughly before taking a sample. This ensures that the sample is representative, and not just an artifact of the particular region of the original mixture from which it was drawn.
- Use an appropriate dilution of the mixture with regard to the number of cells to be counted. If the sample is not diluted enough, the cells will be too crowded and difficult to count. If it is too dilute, the sample size will not be enough to make strong inferences about the concentration in the original mixture. Naturally, a rough idea of the concentration must be known before beginning in order to guess an appropriate dilution. If the mixture is colored, it may be helpful to memorize a particular intensity of that color at which the mixture tends to be easy to analyze.
- Analyze multiple chambers. By performing a redundant test on a second chamber, the results can be compared. If they differ greatly, the method of taking the sample may be unreliable (e.g., the original mixture is not mixed thoroughly). Take the average of the results for a more accurate calculation.
- Make sure to put enough liquid on the instrument that some leaks out of the cover glass when it is placed over the chamber. Otherwise, it is uncertain whether the space under the cover glass is completely filled with liquid. This volume should be the same every time the instrument is used.
- Do not use a paper wipe to dry the excess liquid. The same capillary action that filled the chamber will then dry it out. If using trypan blue, rinse the hemocytometer with distilled water to remove the dye and allow it to dry. Methylated spirits or alcohol (preferably ethanol) may also be used to cleanse the hemocytometer, and it is safe to use lens tissue to wipe the excess away, as long as great care is taken not to warp the hemocytometer grid. For this reason, it is not safe to autoclave (sterilize) a hemocytometer however sterilization should not be essential.
- Watch out for the objective lens. Remember that the hemocytometer is thicker than a normal microscope slide. If focused too closely, the objective lens may contact the instrument. This may affect the choice of objective lens used.
- Count across the rows or down the columns. Use the gridlines to help remember which areas' cells have already been counted.
- There is no need to count the whole chamber. If there are lot of cells, perform a count in a section of the chamber and use the grid to determine what proportion of the chamber that is. Then extrapolate to estimate how many cells are in the chamber, and use that figure in the final calculation. This gives speed at the expense of potential accuracy; if possible, using a more appropriate dilution is better.
- Are the lines in or out? Some cells inevitably fall on top of the outside gridlines that mark the edges of the chamber. The usual practice is to include cells overlapping the top and left lines, but not those overlapping the bottom or right lines - this has the advantage of eliminating redundant counting if adjacent regions are counted.

References


Categories: Laboratory equipment

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  Wikipedia® is a registered trademark of the Wikimedia Foundation, Inc., a non-profit organization.
The total number of cells that were counted in ALL squares that were counted.

\[
\left( \frac{\text{Number of Cells}}{\text{area counted (mm}^2) \right) \times \text{Dilution Factor} \times 10,000 = \text{Number of Cells / mL (cells / mL)}
\]

Indicates where a small volume was taken and put into a larger volume to make less concentrated.

Dilution Factor = \( \frac{\text{Total Volume of (sample + diluent)}}{\text{Volume of sample}} \)

This is the total size of the area to be counted in mm\(^2\).

- a. 1 mm\(^2\)
- b. 0.0625 mm\(^2\) (16 squares = 1 mm\(^2\))
- c. 0.04 mm\(^2\) (25 squares = 1 mm\(^2\))
- d. 0.0025 mm\(^2\)

Formula to calculate cells/mL using Improved Neubauer Hemocytometer grid
Laboratory 5 Directions:

These are the instructions for the lab and lab report. The section of the lab report in which each part should be addressed is indicated in the heading or instructions. Instructions for the graphs and table in the results section is described in PART VI. The questions in PART VII do not need to be answered in lab, but **must** be answered in the discussion (in paragraph form. Do not number, should be like a written journal communication). **Post your lab results on the chalkboard before you leave lab.**

PART I: Observational Results

1. Go to your wine sample. Note any change in appearance (in color, scent, etc) of the culture from Day 1. Indicate whether you see bubbles rising in the flask and if there is froth (bubbles at top) present.

PART II: Information for Methods Sections. *For your methods section you will need to identify the information indicated below. Use this information to address questions in discussion (PART VI of Lab 5).*

1. List **ALL** the **ingredients** in **order** of the grape juice. Why is the order of the ingredients important?

2. Look at the nutrition and front label of the bottle.
   a. What is the **volume (in L)** of grape juice in the bottle?

   b. Identify the **total carbohydrate** in **grams**.

   c. Identify the amount of **sugar** in **g**.

   d. You need to calculate the TRUE starting molar concentration. Picking either b or c as the mass and using the molecular weight of sugar from the previous lab (Lab 4), what is the **starting molar concentration**. (b/c)

   e. Based on your answers to the above questions
      i. Is 0 M TRULY 0 M? (0 M = zero molar)

      ii. If not, explain why and indicate what the 0M solution is representing and how is it still a useful control?
PART III. Measure and report the % change in the amount of alcohol in your sample.

1. Measure a plain water sample using the vinometer and record the results in your notebook. This is to confirm that your vinometer measures the liquid similar to previous measurements. Note any difference between the samples.
2. Record the appearance and CO₂ production of your sample. (PART I)
3. Dismantle your wine and pour it into the provided plastic beaker. Do not disturb the sample for at least 10 minutes to allow it to settle.
4. Measure the % alcohol of your wine (juice + sugar) sample using the vinometer and record the results.
5. Calculate the percent (%) change in ethanol production. To calculate the % change, see the attached instructions.

PART IV. Measure and report the change in the number of cells in your sample.

1. Dilute your wine sample 1:10 in EXACTLY the same manner as you diluted your sample in Lab 4 (the lab prior to this)
2. Using the hemocytometer provided, have a member of the group (who did not load the sample before) load 10µL of your wine sample at each end of the hemocytometer
   a. For each member of the group, count the number of cells using EXACTLY the same protocol that was used in Lab 4. (Same size square(s), same area)
3. Calculate (cells/mL) using the same formula from Lab 4
4. Calculate the % change in the number of cells/mL of YOUR SAMPLE using the formula provided. Record your calculations on your results worksheet. Your initial measurement is your final calculation of the number of cells/mL in the wine sample.

PART V: ABSOLUTELY BEFORE YOU LEAVE

1. Write your FINAL results on the chalkboard!
2. Make sure I have received either an emailed or handed-in version of the initial measurements of your group. If not, give them to me before you leave!

---------------------------------You have completed the data collection for today---------------------------------
PART VI: Graphs to be made for results section. Graphs should be made using class data. All results and analysis should be completed using the class data.

Graph 1. % change in the alcohol percentage (or content) of all the samples. X-axis will have the concentration of sugar, with the Y-axis showing the percent change. You may use either bar or line graphs.

Table 1. CO2 production. You will have observations for Day 0, Day 4, and Day 7 for each of the conditions. Indicate if there bubbles were present? Was culture actively bubbling? Note any change in appearance.

Graph 2: % change in cells or cell growth in response to additional sugar. The X-axis will have the concentration of sugar, with the Y-axis showing the percent change in growth.

PART VII: Questions to be answered in your discussion. You have questions from the first lab that are to be answered in the Introduction. The following questions must be answered in the discussion session of the introduction. All of these questions should be answered using the full class data.

Vinometer measurements.
1. Are vinometers a completely reliable measuring tool for alcohol content? Explain your reasoning. This was the purpose of your plain water measurement, since we know this answer should be zero.

2. Can sugar content interfere with vinometer measurements? Is more sugar likely to increase or decrease the vinometer measurements? You will need to research outside material to answer this question correctly.

3. Based on the class results, explain how the amount of sugar affected the production of ethanol and carbon dioxide. What does this tell you about the amount or rate of fermentation?

Cell counts
4. As the sugar content of the grape juice increased, how did this affect the % change in the number of cells that were present?

5. Based on the class results, explain how the concentration of sugar affects the proliferation of yeast (proliferation = cell division)? How does this relate to the energy needs of a cell during cell division?

6. Using reputable sources, compare the rate of cell growth during fermentation compared to when oxygen is present and hypothesize when cell division would be faster. How could your experiment be altered to test your hypothesis?
Math Central

Quandaries & Queries

Question from Galland:

I am working on graphs and I need to find the percentage of increase from one figure to another. How do I do that? For instance staff hours for 2006 were 28011 and for 2007 were 31230. How do I find the percentage of increase?

Hi Galland.

Percentage change is calculated this way:

$$\text{Percent change} = \frac{\text{later} - \text{earlier}}{\text{earlier}} \times 100\%$$

The sign indicates whether it is a decrease or increase. For your example,

$$\text{Percent change} = \frac{31230 - 28011}{28011} \times 100\% = \frac{3219}{28011} \times 100\% - 0.115 \times 100\% = 11.5\%.$$  

Since this is a positive figure, it is an 11.5% increase.

Hope this helps,
Stephen La Rocque.

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*Math Central is supported by the University of Regina and the Imperial Oil Foundation.*