Yeast viability for fermentation and ethanol production

Why are living yeast cells important for the fermentation process? How can you determine the percentage of viable (living) cells in a yeast culture?

Background

Yeast are single celled organisms that consume simple sugars and produce carbon dioxide and ethanol (alcohol) in the process known as fermentation. For every molecule of glucose that yeast consume, they produce two molecules of ethanol and two molecules of carbon dioxide as seen in the equation below.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

glucose — 2 ethanol — 2 carbon dioxide

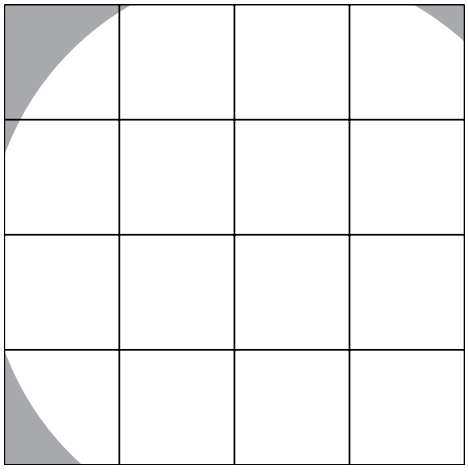
Humans use the fermentation process in food (bread rising, probiotics) and fuel production (ethanol). In order to produce these products it is important to maintain a viable yeast population. One way to check for viable yeast cells is to use a methylene blue solution. When methylene blue is added to a yeast sample, non-viable yeast cells will be stained dark blue.

Materials

- Microscope slides (with gridlines)
- Methylene blue dye
- Test tubes
- Test tube rack
- Disposable pipets or micropipetter with tips
- Compound microscope
- Corn mash samples from ethanol lab
- Safety glasses
- Nitrile gloves

Instructions

1. Prepare a 0.1% Methylene Blue dye solution by adding 0.1g of dye with 100mL of distilled water (This will be enough for several lab groups). *Safety note: Methylene blue is toxic. Wear gloves and safety glasses.*
2. To perform a viability count of mash sample, mix 1mL of mash sample with 1 mL of 0.1% methylene blue solution in a test tube.
3. Mix well by inverting the test tube slowly or using a vortexer, then let it sit to react for one minute.
4. After one minute, add a drop of the dyed mash solution onto a microscope slide that contains grid lines or a disposable hemocytometer to count all the yeast cells visible in the grids. Record total number in a data table or lab notebook.
5. Then count the number of the dark blue cells (these are the dead cells) and record the value in a data table or lab notebook.
6. Create or add to the grid pattern in the diagram below to model your slide. Draw in your yeast culture and include both live (Viable=translucent) and Dead (Non-Viable=dark blue) cells. Record your data in the chart below.



Total yeast cells	Viable cells	Non-viable cells

7. Calculate the percentage of viability using the following equation:
viability [%] = (total cells – total non-viable cells) / total cells × 100

8. What is the percentage of yeast viability in your mash? If yeast typically live for 24 hours, how can you propagate your culture to continue the fermentation process?

9. What are some possible reasons for a low yeast viability percentage in your mash? Research rapid rise yeast to determine their range of tolerance for viability. How can you improve your yeast viability percentage?