Modeling Wine Fermentation

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Video III: Metabolism

In the conversion of sugars into metabolic energy, yeasts and many bacteria produce ethanol instead of detoxifying it like we do. Sugars are broken down and used for energy, forming ethanol as the waste product, which is excreted by the cell. Grapes, with both low pH (2.8-3.8) and high sugar concentration (160 g/L to 240 g/L depending on variety and age), provide an ideal environment for the fermentative yeasts that are among the microbes found on the skins of grapes. Once the grapes are crushed, fermentation is probable, although a number of environmental variables can alter the process.

- Make a list of four variables you suspect would impact the fermentation of grapes.

- Assisting grape fermentation for the manufacture of wine has been a human endeavor since 6000 BC in Mesopotamia. Describe two procedures found in a modern winery that help ensure the success of the fermentation process. Be sure to identify the variables being controlled.

Figure 1. Wild populations of yeasts and bacteria thrive on the grapes as they are growing. When the fruit over-ripens, breaks in the skin allow these microbes entry and fermentation follows.

During fermentation, yeasts metabolize grape sugar for energy, producing carbon dioxide and ethanol as by products. The ethanol is transported rapidly outside of the yeast cells as a waste product. The yeast population grows quickly, grape sugar decreases, and ethanol accumulates to a level that begins to stress the yeast cells. In a closed batch process, the accumulation of ethanol eventually inhibits further microbial action and fermentation stops even though there may be sugar remaining.

- Before drawing blood samples, a phlebotomist swabs the insertion site on the patient's arm with an alcohol pad. Why?

We will further explore fermentation on the computer by:
- creating a simple model of wine fermentation,
- running a simulation with this model,
- carefully considering the results of this fermentation model, and
- investigating further "what if" questions by modifying the model.

Construct the Wine model

We will construct a simple model of wine fermentation using a modeling application called *Extend*. In an *Extend* model, the elements of the system are

represented using picture icons and the flow of energy or products between the elements are represented using connecting lines. To create a model in *Extend* you add the appropriate icons to a worksheet and then connect them to create a system that is a simplified model of the real life system that you are studying. The mathematical equations that actually model the behavior of the wine fermentation system have been worked out in advance and are contained within the "blocks" that the picture icons represent.

The instructions below describe how to create the wine fermentation model. For more detailed instructions on creating, opening, running, saving, and modifying models see "Getting Started with *Extend*" on the *Microbes Count!* CD. The completed wine model is also available in the file called Wine.mox.

1. We are using a version of *Extend* called *Extend LT*. Start the *Extend LT* application by double-clicking on the application icon. The installer for the *Extend LT* application is in the Extend folder on the *Microbes Count!* CD.

2. Use File on the menu bar to Open the wine fermentation worksheet called WineWork.mox (located in the WineFermentation folder on the CD.)

3. The Wine worksheet opens up with an icon for a Plotter, which will be used to graph the results of your simulation.

4. To construct your model of wine fermentation, you will need to add icons for Grapes (grape sugar), Yeast, Ethanol, and Inhibition. To add the icons to your model, click and hold on the Library menu in the top menu bar. In the list of library files, move your mouse to WineLib.lix.

5. You will see a list of the icons available for this model. To put grapes into your model, click on the Grapes item. The Grapes icon will appear on your screen. You can click and drag the icon to arrange it on the worksheet. The cursor will become a hand when you drag an icon.

6. Add the Ethanol, Yeast, and Inhibition icons to the screen in the same way. You should arrange the icons so that your model looks similar to the model in Figure 2.

Figure 2. The completed wine fermentation model.
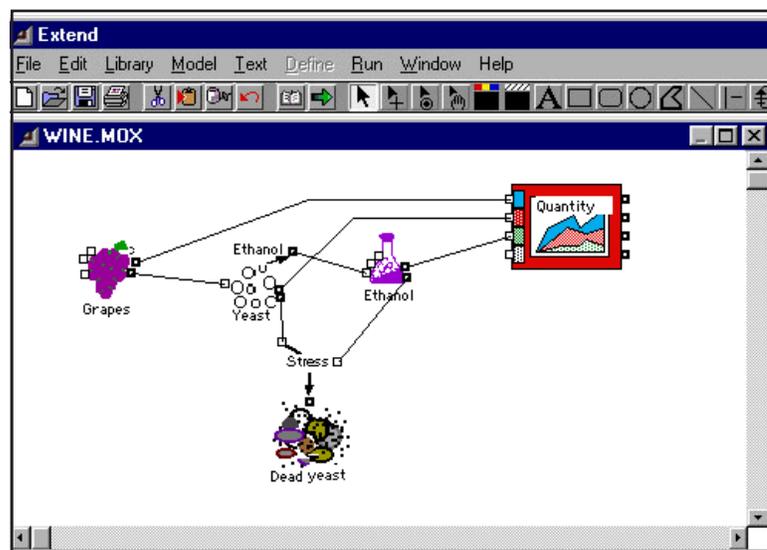
7. Each icon has connectors to attach it to other icons in your model (see the icons in Figure 3.) The small open box on the left of the picture icon is for the flow of energy or products into the icon, such as Grapes into the Yeast. The dark box on the right of an icon represents the flow of products or energy out of the icon, such as Ethanol from the Yeast. The Plotter has four boxes along the left side; it can keep track of four sets of changes in different units and draw four lines on the graph.

Figure 3. Drawing a connection from the output box of the Grapes icon to the input box of the Yeasts icon. When the mouse is held over a connector box, it becomes a "pen" for drawing.

8. Now that you have added all of the icons to your worksheet you need to make the connections between them. With the mouse button held down, draw a line to connect the flow from the Grapes to the Yeast. As illustrated in Figure 3, the line goes from the right connector by the Grapes icon to the left connector of the Yeast icon.
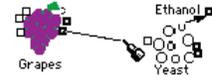
   When you have successfully drawn a line between two connectors, the line becomes bold. To test the connection, drag the icon you just connected to. If the connection line follows, it is attached. If the line is not connected, delete it and try again. To delete a line or an icon, click on it to select it and then press the delete key.

9. Connect the Grapes, Yeast, Ethanol, and Inhibition (stress) icons to model the fermentation process. To make the program graph the changes in the amount of Grapes (or grape sugar), Yeast, and Ethanol you will also need to connect each to an output box left of the Plotter.

When you are finished your model should look like the model in Figure 2. Be sure you have connected the correct boxes; otherwise your model may not run properly. The completed model is also available in the file called Wine.mox.

Run the simulation

Choose Run on the menu bar and highlight Run Simulation. A graph similar to the graph in Figure 4 appears. Grape sugar is scaled on the left vertical axis; yeast density and alcohol are scaled on the right vertical axis, referred to as Y2. Please note that at peak fermentation, there are between 1×10^6 and 25×10^6 yeast cells per milliliter in this model. The density value serves as an indicator for relative population growth.
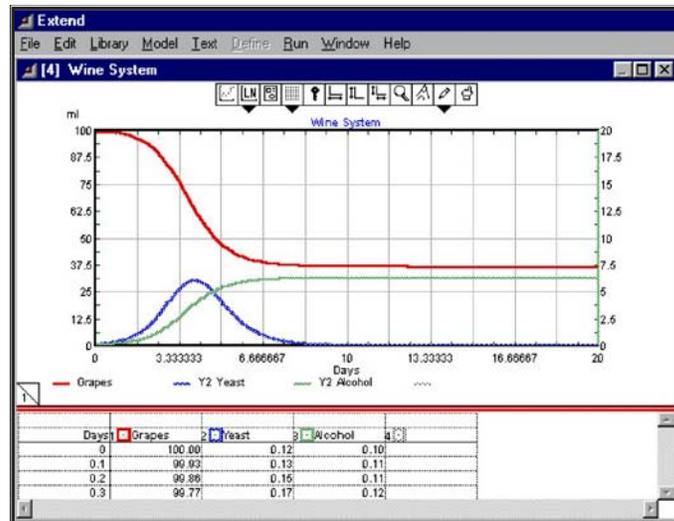

Figure 4. The results from running the wine fermentation simulation.

- Enter Day 4 and Day 5 values for grape sugar, yeast density, and alcohol in the table below.

  Note: You can scroll through the table below the graph or read quantities from the graph itself. To read quantities from a graph, move the mouse across the graph to the point you want to read. Then look at the top row of the table below the graph. For example, if you move the mouse across the graph to the Day 4, you can look at the top row of the table to see the values. Repeat for Day 5.

|	Grape Sugar (ml)	Yeast Density	Alcohol (ml)
Day 4			
Day 5			
Comments			

- Are the values higher or lower on Day 5? Briefly explain why in the comments section.
- Does any grape sugar remain on Day 15? If so, how could you change in the model to use more of the grape sugar up?

Modify the model

One important distinction between wild yeasts and cultivated wine yeasts is their level of alcohol tolerance. Wild yeasts can tolerate about 4% alcohol. Then, as the alcohol concentration increases, fermentation slows down and stops. Tolerance to alcohol is higher in cultivated wine yeasts–up to 14%.

To simulate the improved alcohol tolerance of cultivated yeasts, you can reduce the stress of ethanol on the yeast population. To do this, double click on the Inhibition icon (see Figure 5.) Highlight the stress factor and enter 0.03 in the dialog box.

Figure 5. Modifying the wine model for reduced inhibition.
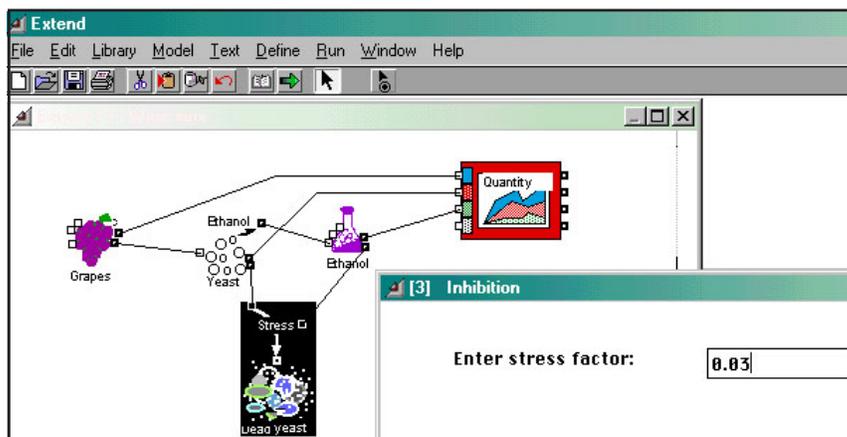
- How will the yeast population respond to this change?

- What will happen to the grape sugar and alcohol levels in the wine?

Run the revised model and compare the results to your predictions.

- Were your predictions accurate?

Explore more with the model called WineYeasts

In this model, two vats of grapes will be fermented under the same conditions at the same time, but with different yeasts (Figure 6.) The first vat, with untreated grapes, will simulate the use of a mix of wild yeasts for fermentation. The second vat, where sulfur dioxide was added early in the wine production to eliminate the wild yeasts, will simulate the use of *Sarccharomyces ellipsoideus*, a cultivated wine yeast with higher alcohol tolerance.
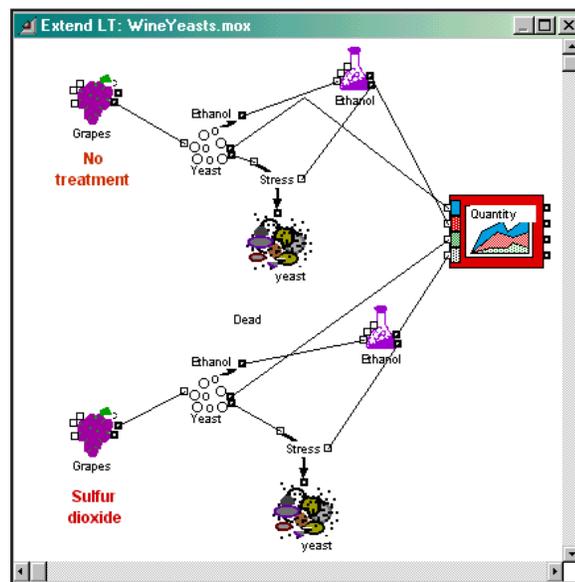

Figure 6. The WineYeasts model represents two vats with treated versus untreated grapes.

- Which yeasts will survive longer?

Now run the simulation and examine the graph and table to determine when the yeast population begins to decline. Note the total ethanol production.

- How could you use a similar model to convince winery owners to use the commercial yeasts your company makes?

Optional activities:

- Problem 1. Unlike the use of sourdough starters, "re-pitching" yeasts (saving yeasts from one batch to be re-used in another) is discouraged.

  Why is the first encouraged and the latter thought to be risky? You may want to look at the "Sourdough Symbiosis" activity in Chapter 2. (Hint: Explain the environmental factors that are less controlled in the latter.)

- Problem 2. In the model, you observed the relationships between grapes, yeasts and alcohol and discovered that the accumulation of a by-product (ethanol) inhibited further conversion of a substrate (grape sugar). The ethanol acts as a toxic waste product which eventually kills the yeasts. However, there are many metabolic pathways in which inhibition of a substrate conversion occurs without killing the organism involved.

  Use your text or other reliable resource to identify:

  1. A by-product found in another metabolic pathway which inhibits the conversion of a substrate without killing the organism involved.

  2. A toxic waste product and the organism that produces it.

Software Used in this Activity

Wine Fermentation

Howard T. Odum (University of Florida) and Elisabeth C. Odum (Santa Fe Community College)

Platform Compatibility: Macintosh and Windows

Additional Resources

Available on the *Microbes Count!* CD

Software

Wine Fermentation

Text

A PDF copy of this activity, formatted for printing

"Getting Started with *Extend*"

Related *Microbes Count!* Activities

Chapter 3: Modeling Wine Fermentation

Chapter 7: Microbiology of Stratified Waters

Chapter 8: Exploring Microbial Fermentation with Korean Kimchee

Chapter 10: Making Sense of Complex Life Cycles: Simulating Toxic Pfiesteria

Unseen Life on Earth Telecourse

Coordinates with Video III: Metabolism

Relevant Textbook Keywords

Ethanol, Fermentation, Metabolic Pathway, Substrate

Related Web Sites

American Home Brewing Supply - YEAST
  http://www.redkart.com/cgi-bin/brew/ahbs/showstory.pl?s=newsletter&a=brew_palette_2

Microbes Count! Website
  http://bioquest.org/microbescount

Office of Energy Efficiency and Renewable Energy
  http://www.ott.doe.gov/biofuels/fermentation_background.html

Unseen Life on Earth: A Telecourse
  http://www.microbeworld.org/htm/mam/is_telecourse.htm

Yeast
  http://www.brewersworld.co.nz/Information/gervin.pdf

References

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Evaluation of Yeast Viability and Concentration during Fermentation Using Flow Cytometry
  http://www.bdbiosciences.com/immunocytometry_systems/application_notes/pdf/23-6289-01.pdf

Fermentations: Problems, Solutions and Prevention
  http://www.uark.edu/depts/ifse/grapeprog/ articles/geis95wg.pdf

Henick-Kling, Thomas. Microbiology of Winemaking
  http://www.nysaes.cornell.edu/fst/faculty/acree/fs430/lectures/thk05fermentation.html

Odum, H. T. and E. C. Odum (2000). *Modeling for All Scales, an Introduction to Simulation*. Academic Press, San Diego, CA.

Odum, H.T. and E.C. Odum (2001). *Wine Fermentation*.

PDB Newsletter: Alcohol Dehydrogenase
  http://www.rcsb.org/pdb/newsletter/2001q1/mom.html

Wine Timeline
  http://www.history-of-wine.com/html/timeline.html

Figure and Table References

Figure 1.   Courtesy Ethel D. Stanley

Figure 2.   Screen shot from *Wine Fermentation*

Figure 3.   Screen shot from *Wine Fermentation*

Figure 4.   Screen shot from *Wine Fermentation*

Figure 5.   Screen shot from *Wine Fermentation*

Figure 6.   Screen shot from *Wine Fermentation*

Figure 7.   Screen shot from *Wine Fermentation*