

FOOD SAFETY



MICROBIAL GROWTH SIMULATION PROGRAM APPLICATIONS

BACKGROUND

The **Microbial Growth Simulation Program** is an adaptation of a computer program developed by the USDA to aid government regulators and food manufacturers in estimating growth characteristics of organisms under varying conditions.

There are many factors that influence bacterial growth. The acronym **FAT TOM** stands for **F**ood composition, **A**cidity, **T**ime and **T**emperature, **O**xygen, and **M**oisture. Although each parameter is often studied individually, it is important to acknowledge that these parameters interact, subsequently changing microbial growth within the food environment. Often, these interactions are very difficult to predict and can be laborious to evaluate in the laboratory.

We developed the **Microbial Growth Simulation Program** to be used as a “dry laboratory” by middle and high school students. Students have the opportunity to change the **temperature**, **available water** (by adding salt, NaCl) and **acidity** of the growth environment and observe how microbial growth changes with **time**. The only portions of **FAT TOM** that are not included in this simulation are **food composition** and **atmosphere (oxygen)**.

USING THE MICROBIAL GROWTH SIMULATION PROGRAM

This program is intended to be used as a hands-on computer simulation laboratory by students, but could also be used by an instructor with a computer projector to stimulate discussion within the class.

The screen displays a graph depicting bacteria count as \log_{10} CFU/g over time with a choice of:

- 3 organisms: *E. coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*
- 3 growth parameters: temperature (°F), pH and salt (% NaCl)
- 4 possible time frames for growth: day, week, month, 3 months

When the program is first opened, the most hospitable conditions of growth will be displayed for each organism. Students can then change the temperature, change the pH, and increase the salt concentration to learn about how these factors can influence bacterial growth. To ease comparison of different conditions or organisms, we suggest that students print a copy of each graph.

Teachers can use this program to teach basic science and math concepts involved in microbiological growth curves.

Once students are comfortable with these concepts, it can be used to allow students a hands-on opportunity to use critical thinking and inquiry-based learning by simulating how changes in temperature, pH, and salt content influence the rate of bacterial growth over time.

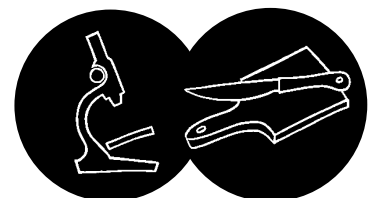
SUGGESTED USES FOR TEACHING BASIC SCIENCE AND MATH CONCEPTS

Bacterial growth.

The **Microbial Growth Simulation Program** can be used to discuss how bacteria grow along with the different sections of a bacterial growth curve. The sections of the growth curve that can be seen are the:

- lag phase,
- logarithmic phase,
- and stationary phase.

During the **lag phase** cells are adjusting to their new environment, repressing or inducing enzyme synthesis and initiating chromosome replication. The length of the lag phase is dependent on the parameters in the program. During the **logarithmic or exponential phase** of growth, bacteria multiply by binary fission (1 cell divides by binary fission to 2 bacteria, that then divide into 4, that divide into 8 etc. Bacteria divide by binary fission regardless of the number of bacteria. Therefore, if you start with



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10,000 bacteria, they would divide into 20,000 that divide into 40,000 etc.) The rate of division (or steepness of the curve) will be dependent upon the conditions set in the program.

The **stationary phase** occurs when the bacterial level reaches a critical concentration and in this program it is always set at 1×10^9 (yes, that's 1 billion). Although no longer growing, bacteria at this stage turn on very different sets of genes and usually are more resistant to stress than when in the exponential phase. This program does not include or address the death/decline phase.

Logarithmic numbers.

- The bacterial data in this program are presented as log₁₀. When plotted in this manner a data point at 6 on the y-axis represents 1×10^6 or 1,000,000 bacteria, 4 represents 1×10^4 or 10,000, and 2 represent 1×10^2 or 100 bacteria.

- You can ask students to speculate why the data are presented in this manner. Microbiological data are presented in this manner since bacteria grow logarithmically. It is impractical and difficult to plot between 1000- 100,000,000 on a linear scale on a normal piece of paper.

Definition of a "colony forming unit" or "CFU".

- Bacteria grow as individual cells or clumps. Microbiologists enumerate numbers by plating on agar plates and it is impossible to know if a colony grew from either individual cells or clumps.

- Therefore, microbiologists present count data as "colony forming units," to acknowledge that the levels may be underestimates of actual levels if the colonies grew from clumps.

Using graphs slopes to evaluate "rate."

- The logarithmic or log phase of growth is where the bacteria are multiplying logarithmically. During log phase growth, the slope of the line (change in cell numbers divided by the change in time or $\Delta y/\Delta x$) is an indication of the bacterial growth rate.
- Emphasize that the point for calculation should be in the middle of the logarithmic phase, rather than at the beginning and end of the growth. The steeper the line upward, the higher the slope and the faster the bacteria are growing.

- A sample exercise would be to ask students to determine at what temperature the bacteria grow the fastest by calculating the slope during the log phase of growth at three different temperatures (40, 72, and 98.6°F). To do this, students should keep the level of NaCl and pH constant but change the temperature. Have the students print the three graphs to help with data collection.

Calculation of k and Generation time. (*This exercise is a little more challenging but may be appropriate for AP courses.*)

This activity is similar to the previous exercise, but rather than using the slope of the log phase of growth, microbiologists calculate k or number of divisions that occur per unit time it takes for a cell to double using the following formula.

Ask students to determine at what temperature the bacteria grow most rapidly by calculating k or **generation time** (amount of time required for cell division) during the log phase of growth at three different temperatures (**40, 72, and 98.6 °F**), keeping the level of NaCl and pH constant but changing the temperature. Have the students print the three graphs to ease data collection.

This is done using the following equation:

Where k = number of generations/unit time
 N_a = number of cells (CFU/g or ml) at time = a
 N_b = number of cells (CFU/g or ml) at time = b
 t = change in time $t_b - t_a$
 0.301 is a conversion factor to convert log₁₀ to ln

NOTE: This program presents data as "log₁₀ CFU/ml". Students can read the cell numbers directly off the graph and do not need to do the log₁₀ conversion.

The inverse of k ($1/k$) = **Generation time** or the amount of time required for one cell to double

$$k = \frac{\log_{10} N_b - \log_{10} N_a}{0.301 t}$$

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SUGGESTED USES FOR TEACHING FOOD SAFETY CONCEPTS

The "two hour rule" is a broad consumer food safety guideline based upon all foodborne pathogens and assumes that the product is readily permissive to bacterial growth. Not all bacteria are familiar with this rule and, depending on the bacteria, growth may slow at higher temperatures than the "danger zone" cut-off of 40°F. Growth of bacteria is important since the higher the number of the pathogen present, the more likely people are to become sick. However, it is just as important to emphasize that **lack of bacterial growth does not equal safety**. If pathogenic bacteria are present and not growing, the food product may still cause foodborne disease.

LEAVING FOOD AT ROOM TEMPERATURE.

Ask students to put the acidity and salt levels at the most hospitable (pH 7 and 0.5% NaCl) and set the temperature at room temperature (see Table 1 for temperatures). Have the students examine the growth of each organism over 1 day and 1 week.

- Have the students discuss how long it takes for each of the organisms to get to stationary phase and how this relates to the "maximum of 2-hour holding at room temperature" rule in safe food handling.
- How long does it take to get to stationary phase at 22°C (72°F) if the conditions are changed to the least hospitable (the maximum % of salt and pH 4.5)?

LEAVING FOOD AT REFRIGERATED TEMPERATURE

Have the students do the same exercise as above - (hospitable conditions with 0.5% salt and pH 7) - except have them change the temperature to 4°C (39°F).

- Ask them to look at the growth over 1 day, 1 week, 1 month, and 3 months.
- Ask them about the amount of time foods can be kept under refrigeration.

How do conditions influence microbial growth?

- Ask students to set temperature and salt levels at most hospitable. Ask the students to predict what will happen when the pH is lowered.

- Ask students to slowly decrease the pH and describe how the stages of microbial growth are influenced.
- Have them bring the pH back to 7 and repeat the exercise by slowly increasing NaCl. Have them put back the level of NaCl to 0.5% and have them slowly decrease the pH. If multiple parameters are changed, are the predicted growth curves different?
- One exercise would be to challenge the students to find a combination of conditions that will inhibit growth of all three organisms for 3 months.

Do the different bacteria species grow differently?

The answer to that question is yes. The food holding temperature rules are based upon foodborne pathogenic bacteria that grow at extremes (high and low growth temperatures). This program will allow students to see that bacteria grow very differently.

- *Staphylococcus aureus* grows very slowly at temperatures below room temperature (approximately 70°F).
- *Listeria monocytogenes* and *E. coli* O157:H7 both can grow fairly quickly at room temperature and will continue to grow (at a slower rate) at refrigeration temperature.
- On the other hand, *S. aureus* and *L. monocytogenes* have reputations for growing in high salt, reduced water conditions.

Table 1. Commonly used temperatures in food safety.

	Degree Fahrenheit	Degree Celsius
Freezing point of water	32°F	0°C
Refrigeration temperature	Above 32 -39.2°F	Above 0 - 4°C
"Danger Zone"	40-140°F	4.4 - 60°C
Room temperature	72°F	22°C
Human body temperature		
(often optimal growth temperature)	98.6°F	37°C
Boiling point of water	212°F	100°C

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