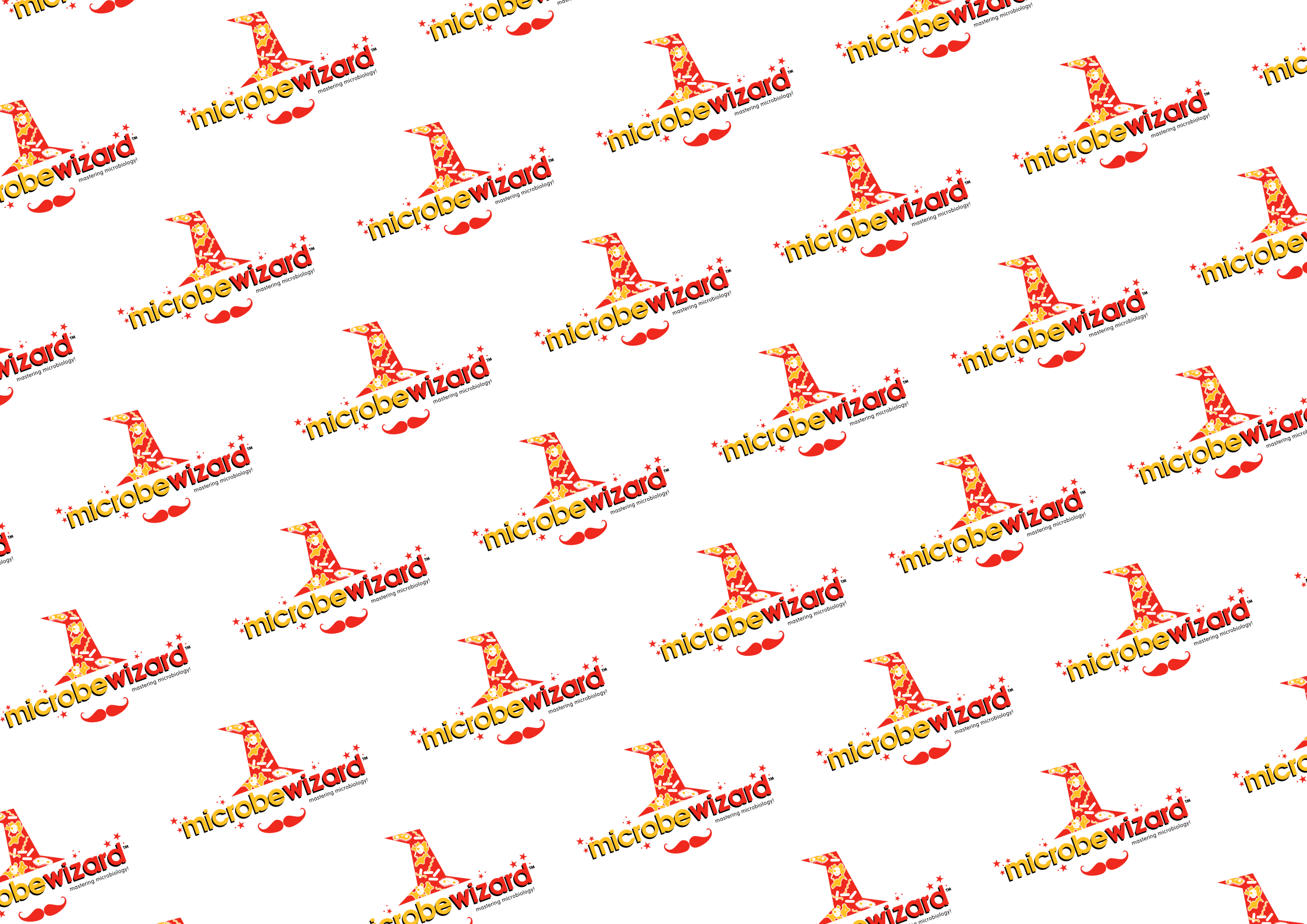
studentguide

Sample Experiment for Bread


microbewizard™
mastering microbiology!

General Information

Counting Microbe Colonies

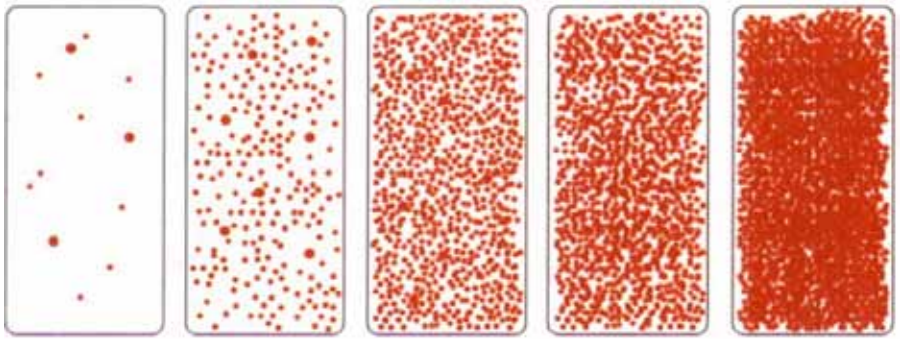


Figure 1 - Counting Panels - Making Total Colony Counts (TCC)

A 10^3 CFU/mL (1 CFU/cm^2)
B 10^4 CFU/mL (10 CFU/cm^2)
C 10^5 CFU/mL (45 CFU/cm^2)
D 10^6 CFU/mL (80 CFU/cm^2)
E 10^7 CFU/mL (100 CFU/cm^2)

NOTES: A colony Forming Unit (CFU) is a visible colony formed from usually one microbe cell or spore. TCC (total colony counts) is the total number of visible microbe colonies (growth areas) on a paddle. Paddle area is 10 cm^2. 10^4 = 10,000; cm^2 = square centimeter (area).

FOR SURFACE COUNTS: Use the corresponding Counting Panel value TCC = (CFU/cm^2)
FOR DILUTION COUNTS: Multiply the corresponding Counting Panel value by the dilution factor (10^2) TCC = panel count x dilution factor (10^2)

Sizing Microbe Colonies

Figure 2 - Sizing Microbe Colonies.
The embedded plastic stars (+) measure 4mm point-to-point. These yeast colonies (*Saccharomyces cerevesiae*) are 1-2mm in diameter. Source: Luis Camus, MD

Microslide® Anatomy

Figure 3 - Microslide® Anatomy.
Source: Luis Camus, MD

Microbes that Like

Bread

Microbe Wizard™ Bread Facts

Figure 1 - Wheat, bread, wheat flour and cookies! Source: USDA

Bread loaf anatomy:
- ***Crust*** - the hard, outer, brown layer
- ***Crumb*** - the inner loaf body

Wheat bread is made of:
- wheat flour (gluten protein, starches)
- water
- yeast (fermentation cell respiration yielding alcohol and CO_2)
- added:
  - fats (to prevent "staleness"-helps retain free-water)
  - salt (helps bind proteins; less dough "stickiness")
  - preservatives (extends shelf life against microbe "spoilers")

Wheat was first planted in the US as a "hobby crop".

Today, an acre in Kansas produces enough bread to feed 9,000 individuals a day.

A bushel of wheat is equivalent to:
- 1 million individual kernels
- 60 pounds (27 kg)
- 60 pounds (27 kg) whole-wheat flour
- 42 pounds (19 kg) white flour
- 42 loaves of commercial white bread
- 90 one-pound loaves of whole-wheat bread

A commercial loaf of bread has:
- 16 oz flour (1.5 lb. / 453 g loaf)
- 24 slices

Factors Influencing Microbial Growth on Breads

Food spoilage is caused by the enzymes produced by microbes. Food spoilage microbes need: organic nutrients (proteins, carbohydrates, fats), a convenient temperature, some moisture, in most cases, some air and a suitable pH.

1. Nutrients
Breads are mostly a mixture of flour and water that is baked. Flour is a ground powder made from cereal grains (usually wheat and corn), seeds, or roots. The caloric content of flour is mostly carbohydrates (80%), proteins (15%), and fats (5%).

2. Moisture Content (water activity)
Available moisture (water) is essential for microbe growth and survival. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (aw) of the food or surrounding environment. The water activity (aw) of a food is the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions. A water activity of 0.80 means the vapor pressure is 80 percent of that of pure water. The water activity increases with temperature. Bread has a water activity of 0.96. If breads are cut or placed into a closed container (e.g. plastic bag) they will "moisture equilibrate."

Microbe Water Activity	
Most Bacteria	>0.91
Most Yeasts	>0.88
Most Fungi	>0.80

3. pH
In their natural state, most foods such as meat, fish, and vegetables are slightly acidic while most fruits are moderately acidic. A few foods such as egg white are alkaline. Breads have a pH range of 5.0 - 6.2.

Microbial cells must maintain their intracellular pH (pHi) above some critical level so that cellular proteins will not denature. Most harmful food microbes can survive within these pH ranges:

Microbes & pH	
Most Bacteria	pH 4 to 9
Most Yeasts	pH 2 to 8
Most Fungi	pH 0 to 11

4. Temperature
The most important factor directly affecting how fast microbes grow. Bread-spoiling microbes prefer a temperature range of 30˚C (86-113˚F) but can grow in temperatures as low as 5-15˚C (41-59˚F). As a rule of thumb, for every increase in temperature of 10˚C (50˚F), the activity increases two times. This rule is true within the temperature range of 32 to 60˚C.

5. Time (Shelf Life)
Bakers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from when the product is produced until the time of consumption. The "sell by" date must incorporate the shelf life of the product plus a reasonable period for consumption that consists of at least one-third of the approximate total shelf life of the perishable food product.

6. Food Additives: Preservatives
Preservatives are added to prevent the growth of unwanted microorganisms, food spoilers and food pathogens. Most bakeries add one or more of the following chemical compounds to their bread products to extend shelf life and reduce microbe contamination:

Preservatives & Microbes

Compound	*Type*	*Antimicrobial Activity*
Citric acid (acid citrates)	acidulant	BACTERIA (++) MOLDS (+) YEASTS (+)
Benzoic acid (benzoates)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)
Sorbic acid (sorbates)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)
Sulfites	preservative	BACTERIA (+) MOLDS (++) YEASTS (++)
Propionic acid (propionates - Ca, Na, K)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)

Figure 2 Moldy rye bread. Can you identify this mold microbe?

Guided Investigation

Investigating Microbes on Breads Using Impression Sampling

Objectives:
- Why is bread a microbe food?
- What kinds of microbes like breads?
- What can reduce microbe populations in breads?

What You Need:
- TSA / RB Microslide® paddle [KIT]
- Bread slice [LOCAL]
- Zip-closure plastic bag [LOCAL]
- Toothpick [LOCAL]
- Magnifier [LOCAL]
- Laboratory Notebook [LOCAL]
- Digital camera (optional) [LOCAL])
- Supervising adult!

What You Do -13 Steps:

SAFETY: Remember to wash your hands after handling bread samples.

1. Take a trip to a local grocery store or bakery. Obtain a baked loaf of bread.
2. In the laboratory, use a toothpick to carefully punch 4-6 small holes in a zip-closure plastic bag.
3. Place a bread slice into a zip-closure bag and seal it. You may want to encourage bread microbes by adding moisture - a moistened piece of paper toweling or a quick spray of the bread slice with a water mister.
4. Observe the bread slice carefully over time (5-9 days) and away from sunlight. Do any visible microbes appear?
5. To direct-sample growing microbes on breads, carefully open the plastic bag so that there is enough room for the paddle to be inserted to sample a contaminated surface.
6. Twist to remove the paddle from the vial.
7. Allow the paddle surface to come into physical contact with the contaminated bread area(s). Contact both paddle surfaces on various contaminated bread areas.
8. Replace the inoculated paddle into the vial.
9. Secure the paddle with transparent sticky tape. Wash your hands!
10. Incubate the paddle for 5-7 days at room temperature away from sunlight.
11. Monitor daily for signs of colony growth - DO NOT ATTEMPT TO REMOVE THE PADDLE FROM THE VIAL. Instead, make all observations through the vial. Use a magnifier to help you observe finer details of your microbe finds!
12. Use the *Bread Micro-Community Guide* and the Counting Panels to presumptively (tentatively) identify bread microbes. Record this data in your Laboratory Notebook.
13. If you have a digital camera (iPad, iPhone or similar), use it to take close-up pictures of microbe colonies for color printing to help in later identification.

Analysis Example
A TSA/RB paddle was applied to the surface of a piece of moldy bread. The paddle was incubated at room temperature (70˚F / 20˚C) for 4 days. Use the *Bread Micro-Community Guide* to identify the bread microbe growing on both the TSA and RB paddle agars:

Figure 3 - Hunting black bread mold.

A TSA/RB paddle was applied to the surface of a piece of moldy bread. The paddle was incubated at room temperature (70˚F / 20˚C) for 5 days. Use the *Bread Micro-Community Guide* to identify the bread microbe growing on both the TSA and RB media. Source: Luis Camus, MD

DATA TABLE 1
Investigating Microbes On a Bread Slice

Paddle Agar	*Tentative Microbe Identification*	*Environmental Conditions / Notes*
RB		
TSA		

Designing Experiments

Remember that an experiment is a process or study that results in data (information). The results of experiments are not known in advance. Experimental design is a process of planning a study (experiment) to meet specified objectives. When designing an experiment, follow the scientific method - a process involving:

- Formulating a question
- Making an educated guess (a hypothesis)
- Testing your guess (experimenting)
- Analyzing your results
- Drawing conclusions (gaining knowledge; being able to explain)

After conducting investigations, you should be able to answer these investigational objective questions:

- Why is bread a microbe food?
- What kinds of microbes like breads?
- What can reduce microbe populations in breads?

More Ideas! (open inquiry)

Try these additional activities using either bread slices or additional Microslide® paddles to capture and sample bread microbes.

1. Microbe Race! Create these experimental bread slice setups: commercial bread slice (with preservatives) against a "home-baked" bread slice (without any added preservatives). Which bread will "win" (see evidence of microbe spoilage) the microbe race?

2. Mold Race 2: Which experimental condition makes it more likely for microbe spoilage to occur - An un-opened loaf of bread, or one purchased at the same time with 10 small toothpick holes poked into it?

3. Do stale (dried out) bread slices provide longer protection against microbe spoiling?

4. Which spoils more rapidly - a slice of white bread or a slice of toasted white bread after being placed in a zip-seal bag?

Questions continued....

5. Which configuration (inside a sealed zip-seal bag) is more sensitive to microbial spoiling - a baked un-cut loaf or a baked sliced loaf?

7. Compare different bread types for rate of microbe spoilage: white, brown, whole wheat-meal, bagel)

8. Compare the effects of temperature on bread microbe spoiling. Use the same type of bread, incubated under identical conditions, at different temperatures (refrigeration, room, warm).

9. How long (past its "Best Used By" date) will a commercial bread loaf (with preservatives) remain "fresh" - with evidence of microbial spoilage?

Going Further

Hunting for Bread Microbes - making sourdough bread starter
Attract yeast microbes by making your own sourdough bread starter - confirm your finds using a Microslide® paddle!

Figure 4 - Sourdough Bread Starter - Day 3
Notice the bubbles of carbon dioxide forming - a sure sign of microbe activity! Source: Kenneth G. Rainis

What You Need:

- Kitchen measuring cup [LOCAL]
- Whole wheat or pumpernickel flour (whole grain flours contain more microbe-friendly ingredients and moisture!) [LOCAL]
- Glass or plastic container (4 cup measure, 240cc) [LOCAL]
- Cool, non-chlorinated water (bottled water) [LOCAL]
- Wooden mixing spoon [LOCAL]
- Handkerchief [LOCAL]
- Popsicle stick (or similar) [LOCAL]
- Sticky tape (roll) [LOCAL]
- Warm incubation environment (68 - 70˚F / 20 - 21˚C) [LOCAL]
- Supervising Adult!
- Unopened (STERILE) TSA /RB Microslide® paddle [KIT]
- Digital camera (optional) [LOCAL]