EFFECTS OF PH ON MICROBIAL GROWTH

Principle and Purpose

The term 'pH' describes the hydrogen ion (H^+) concentration in a solution. H^+ is actually a proton. Hence, pH is a measurement of protons in a solution, the scale of which is logarithmic and inversely related to concentration. If the concentration of protons is high, then the pH is low or often referred to as acidic. Acidic pH is generally considered to be on the scale of 0-5, with pH 0 being equivalent to battery acid and pH 5 essentially equal to that of black coffee. Conversely, if the concentration of protons is low, then the pH is high or referred to as basic (or alkaline). Basic pH is generally considered to be on the scale of 9-14, with pH 9 being equal to a solution of baking soda, whereas pH 14 is near the alkalinity of liquid drain cleaner. pH values in the 6-8 range tend to be less extreme with pH 7 being perfectly neutral.

Microbial species can be found growing in a variety of environments including those having extreme pH levels. For example, known members of Bacteria and Archaea thrive in acid mine drainage having pH values of 3 or less. Such microbes are defined as acidophiles ('acid loving'). Other microbes have adapted to alkaline environments (alkalophiles; also acceptable is the term alkali*i*phile). Yet, the majority of microbes are neutrophiles preferring environmental pH levels of 5.5-8.0. It is notable that each microbial species possesses a definitive pH growth range and a distinctive pH growth optimum (Fig. 1).

One might ask how these organisms survive in such conditions given that extreme pH levels denature proteins, which in turn would dramatically affect cellular metabolism and eventually viability. The answer is that these organisms have evolved mechansims to maintain their cytoplams at near neutral pH, thereby permitting intracellular functions to continue as normal. For example, acidophiles use defenses that enforce the cell membrane against the damaging effects of acidic pH levels. Some form biofilms to slow the movement of molecules into the cell, whereas others secrete buffering molecules to raise the pH (i.e., make it more basic) in the cytoplasm or nearby surrounding environment. Moreover, a number of acidophiles have evolved H^+ pumps that move protons out of the cell, thereby maintaining their internal pH near 7.0.

In the present exercise students will examine how pH affects the growth of three common bacteria (*Escherichia coli*, *Staphylococcus aureus*, and *Alcaligenes faecalis*) and the eukaryote, the yeast *Saccharomyces cerevisiae*. The degree to which pH affects growth of these microbes shall be observed subjectively (i.e., visually) and objectively (i.e., using a spectrophotometer).

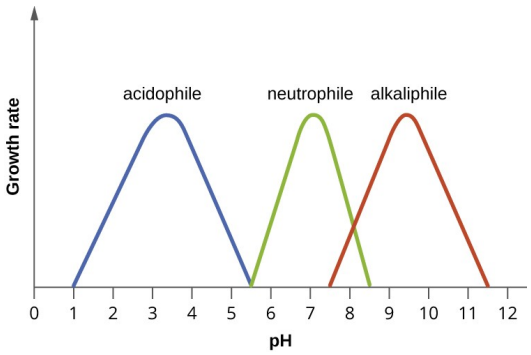


Figure 1. Example pH ranges and growth optimums for the different classes of microbes. (https://courses.lumenlearning.com/microbiology/chapter/the-effects-of-ph-on-microbial-growth/)

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Analyze experimental data to determine the general pH growth range of the selected microbes used in this exercise; and
- Properly operate and generate useful data from a commonly used type of spectrophotometer.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms

- The following organisms should be provided as 24-48 hour-old TSA slant cultures:
  - *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
  - *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]
  - *Alcaligenes faecalis* (ATCC 19018) [abbreviated as *A. faecalis*]
- Sabouraud dextrose agar culture (3-5 days old) of *Saccharomyces kudriavzevii* (ATCC 2601; formerly designated as *Saccharomyces cerevisiae*) [abbreviated as *S. kudriavzevii*]

Materials

- Sterile saline (5 ml) in standard test tube
- Sterile bulb transfer pipettes
- TSB (3 ml) in 13 x 100 mm tubes
- TSB, pH 3.0, (3 ml) in 13 x 100 mm tubes
- TSB, pH 5.0, (3 ml) in 13 x 100 mm tubes
- TSB, pH 7.0, (3 ml) in 13 x 100 mm tubes
- TSB, pH 9.0, (3 ml) in 13 x 100 mm tubes
- Alcohol wipes
- KimWipe™
- Wickerham card

Equipment

- Spectronic 200E Spectrophotometer (Spec 200E)
- Vortex

Procedure

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain four test tubes containing 5 ml of sterile saline. Label one tube as *E. coli*, the second as *A. faecalis*, the third as *S. aureus*, and the remaining tube as *S. cerevisiae*.
2) Obtain four tubes each containing 3 ml of pH-adjusted TSB (pH 3, 5, 7, and 9 in the 13 x 100 mm tubes). Be sure to label each tube with its respective pH.
3) Label one series of pH-adjusted broths (one tube each of pH 3, 5, 7, and 9) as *E. coli*. Similarly, prepare three other series of pH-adjusted broths labeling one as the *A. faecalis*, another as *S. aureus*, and the remaining series as *S. cerevisiae*.
4) Obtain eight tubes each containing 3 ml of non-pH-adjusted ('normal') TSB tubes and label a pair of tubes *E. coli*, the second pair as *A. faecalis*, the third pair as *S. aureus*, and the remaining pair as *S. cerevisiae*.

5) To the saline tube labeled *E. coli*, aseptically transfer a loopful of growth from the TSA culture of *E. coli*. Similarly, aseptically transfer a loopful of growth from the cultures of *A. faecalis*, *S. aureus*, and *S. cerevisiae* to the respectively labeled saline tubes.
6) Thoroughly mix the *E. coli* saline suspension thoroughly by rolling the tube between both palms ten times or more. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that the tube rolls out of the hands causing leakage or breakage.
7) Using a sterile bulb transfer pipette, inoculate the *E. coli*-labeled pH-adjusted broth series (pH 3, 5, 7, and 9 in the 13 x 100 mm tubes) with 0.1 ml of the saline suspension of *E. coli* (Step 5). In addition, inoculate the *E. coli*-labeled non-pH-adjusted ('normal') TSB tube with 0.1 ml of the same *E. coli* saline suspension. Do not inoculate the second *E. coli*-labeled non-pH-adjusted ('normal') TSB tube. The latter will serve as sterility control and a blank for turbidity measurements.
8) Thoroughly mix the *A. faecalis* saline suspension thoroughly by rolling the tube between both palms ten times or more. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that the tube rolls out of the hands causing leakage or breakage.
9) Using a new sterile bulb transfer pipette, inoculate the *A. faecalis*-labeled pH-adjusted broth series (pH 3, 5, 7, and 9 in the 13 x 100 mm tubes) with 0.1 ml of the saline suspension of *A. faecalis* (Step 5). In addition, inoculate the *A. faecalis*-labeled non-pH-adjusted ('normal') TSB tube with 0.1 ml of the same *A. faecalis* saline suspension. Do not inoculate the second *A. faecalis*-labeled non-pH-adjusted ('normal') TSB tube. The latter will serve as sterility control and a blank for turbidity measurements.
10) Thoroughly mix the *S. aureus* saline suspension thoroughly by rolling the tube between both palms ten times or more. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that the tube rolls out of the hands causing leakage or breakage.
11) Using a new sterile bulb transfer pipette, inoculate the *S. aureus*-labeled pH-adjusted broth series (pH 3, 5, 7, and 9 in the 13 x 100 mm tubes) with 0.1 ml of the saline suspension of *S. aureus* (Step 5). In addition, inoculate the *S. aureus*-labeled non-pH-adjusted ('normal') TSB tube with 0.1 ml of the same *S. aureus* saline suspension. Do not inoculate the second *S. aureus*-labeled non-pH-adjusted ('normal') TSB tube. The latter will serve as sterility control and a blank for turbidity measurements.
12) Thoroughly mix the *S. cerevisiae* saline suspension thoroughly by rolling the tube between both palms ten times or more. Roll the tube quickly, but not so harshly that the broth splashes onto the tube cap or such that the tube rolls out of the hands causing leakage or breakage.
13) Using a new sterile bulb transfer pipette, inoculate the *S. cerevisiae*-labeled pH-adjusted broth series (pH 3, 5, 7, and 9 in the 13 x 100 mm tubes) with 0.1 ml of the saline suspension of *S. cerevisiae* (Step 5). In addition, inoculate the *S. cerevisiae* -labeled non-pH-adjusted ('normal') TSB tube with 0.1 ml of the same *S. cerevisiae* saline suspension. Do not inoculate the second *S. cerevisiae*-labeled non-pH-adjusted ('normal') TSB tube. The latter will serve as sterility control and a blank for turbidity measurements.
14) Incubate the various tubes inoculated with *E. coli*, *A. faecalis*, and *S. aureus* at 37°C for 36-48 hours. Incubate the TSB tubes inoculated with *S. cerevisiae* at room temperature (25°C) for 36-48 hours. The uninoculated control TSB tubes should be incubated at the same temperature used for the given microbe.

15) Remove the tubes from their incubation locations. Mix these cultures thoroughly using a vortex operating at a *middle-speed setting*.

16) Observe the degree of turbidity by comparing all tubes in a series to the uninoculated TSB tube incubated at 25°C. The use of a Wickerham card (Fig. 2) may help facilitate these observations.

Note: To help discern the degree of turbidity of each tube, a Wickerham card can be used to compare the degree of growth (Fig. 2). To use this card, hold the tubes to be assessed for turbidity side by side and no more than 1 inch from the face of the Wickerham card. With adequate lighting, compare the appearance/sharpness of the lines on the card as viewed through the tubes. Do not hold the tubes flush against the card.

Figure 2. Wickerham card and various McFarland standards of turbidity. http://www.bioanalytic.de/en/product/article/wickerham-card-539.html

Record your observations on the report sheet attached to this exercise.

Interpretation of Results: Use the following scale to record your observations: 0, no growth; +, little visible growth/turbidity; ++, some visible growth/turbidity; +++, moderate growth/turbidity; ++++, luxurious growth/turbidity. The uninoculated 25°C-incubated TSB tube in a series should be used an example of '+++' growth. The uninoculated (hopefully still not turbid) TSB tube should be an example of '0' growth.

17) Measure the turbidity of each tube in terms of absorbance using a Spectronic 200E spectrophotometer. Set the instrument set at 600 nm and the uninoculated TSB tube as the 'zero' control. Prior to taking a measurement, be sure to thoroughly mix the contents of each tube using a vortex operating at a *middle-speed setting* prior to placing the tube in the spectrophotometer.

Record your observations on the report sheet attached to this exercise.

Note: Instructions for use of the Spectronic 200E can be found at the following URL: http://crcooper01.people.ysu.edu/microlab/Using-Spec200E.pdf.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR VISUAL OBSERVATIONS.

Use the following scale: 0, no growth; +, little visible growth/turbidity; ++, some visible growth/turbidity; +++, moderate growth/turbidity; ++++, luxurious growth/turbidity. Use the 'normal' inoculated TSB tube as an example of "+++' growth.

Organism	Inoculated Media				
	'normal' TSB	TSB, pH 3.0	TSB, pH 5.0	TSB, pH 7.0	TSB, pH 9.0
Escherichia coli					
Staphylococcus aureus					
Alcaligenes faecalis					
Saccharomyces cerevisiae					

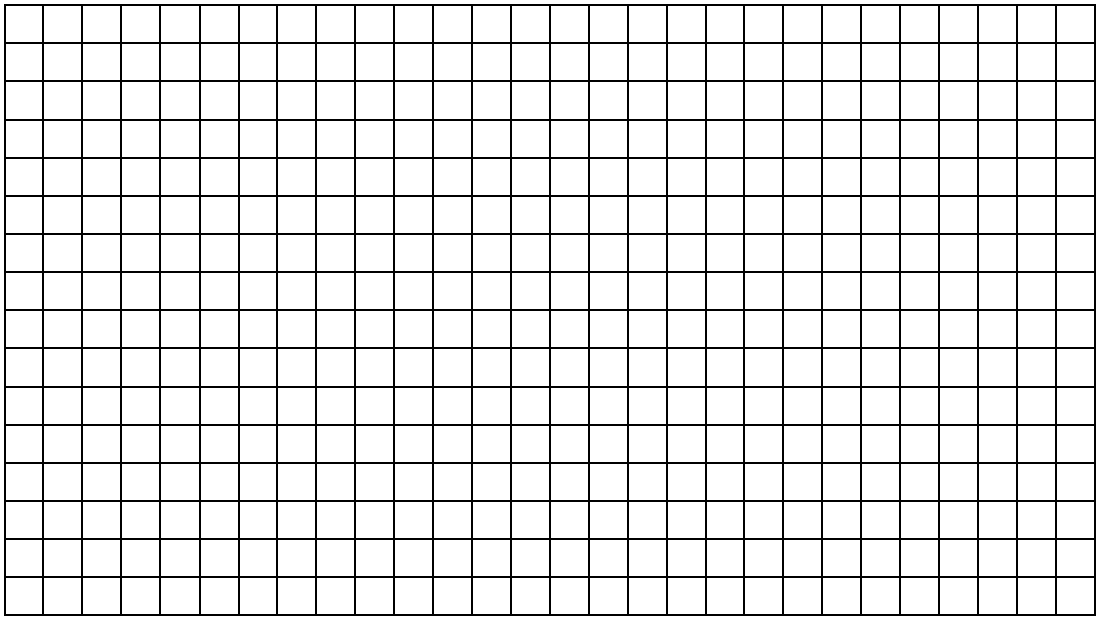
COMPLETE THE FOLLOWING TABLE BASED UPON SPEC 200E READINGS.

Organism/Type of Measurement	Inoculated Media				
	'normal' TSB	TSB, pH 3.0	TSB, pH 5.0	TSB, pH 7.0	TSB, pH 9.0
Escherichia coli					
Absorbance					
% Transmission					
Staphylococcus aureus					
Absorbance					
% Transmission					
Alcaligenes faecalis					
Absorbance					
% Transmission					
Saccharomyces cerevisiae					
Absorbance					
% Transmission					

Note: % Transmission is related to Absorbance as follows: Absorbance = 2 – log(%T)

Student Name: _____

Graph the pH of the medium (x axis) against the amount of growth (absorbance or %T; y axis) for each species examined in this exercise. Use different colored lines and symbols for each species. Be sure to present a figure legend for this graph.



Discussion Questions:

1. Given the data you generated, how would you classify each of the species tested in this exercise with regard to their growth in different pH levels?

   Escherichia coli

   Staphylococcus aureus

   Alcaligenes faecalis

   Saccharomyces cerevisiae

2. Among those species examined, which group (prokaryotic vs. eukaryotic) appeared to be more acid tolerant?

3. How do the subjective (visual) results compare with the objective (spectrophotometer) results of this study?