Utah State University

DigitalCommons@USU

Laboratory Experiences in Mathematical Biology

Open Educational Resources

2016

Yeast Lab

Matt Lewis
Utah State University

Jim Powell
Utah State University

Laboratory Experiences in Mathematical Biology

2016

Yeast Lab

Matt Lewis
Utah State University

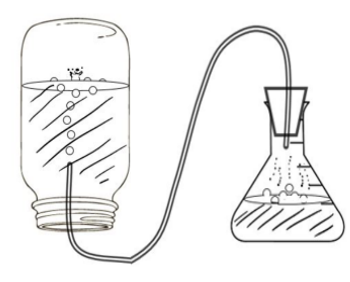
Jim Powell
Utah State University

Yeast Lab

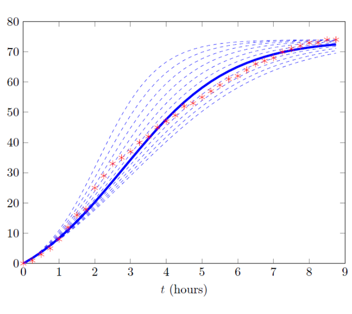
Laboratory Experiences in Mathematical Biology

Overview: Yeast are an ideal candidate for students to model mathematically. As Gause (1934) states ``...yeast cells are sometimes subject to perfectly definite quantitative laws. But it has also been found... their trends often do not harmonize with the predictions of the relatively simple mathematical theory.'' These characteristics allow students to initially engage in modeling yeast dynamics with relative confidence but the students must critically and creatively adjust their models in order to get their predictions to better harmonize with yeast data. The Yeast Lab is usually performed with classes comprised of upper-class undergraduates and graduate students from mathematics, statistics, biology, natural resources and biological engineering.

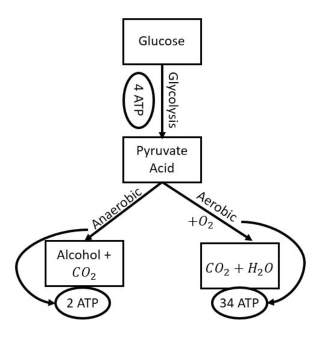
Lesson Outline: The outlined expectations and agenda were originally constructed for a mathematical biology course consisting of mathematics, statistics, biology, natural resources and biological engineering students with calculus and differential equations experience. See Pedagogical Resources for teaching and scaffolding suggestions.



Lab Setup: Yeast is grown in a small, capped flask, generating carbon dioxide which is measured in an inverted jar.



Data and Examples: Data along with some student approaches are presented to illustrate the range of student creativity and to help prepare teachers to scaffold student thinking.



Background and Extensions: To build biological context and facilitate in lab presentation, a brief history of yeast in mathematics is discussed along with a short description of cellular respiration.

Assessment Items: Primary assessment of student learning is taken from students' written reports additional assessment items targeting lab objectives are included here.

Yeast Lab

Laboratory Experiences in Mathematical Biology

Lesson Outline: The outlined expectations and agenda were originally constructed for a mathematical biology course consisting of mathematics, statistics, biology, natural resources and biological engineering students with calculus and differential equations experience. See Pedagogical Resources for teaching and scaffolding suggestions.

Expectations: Teams are expected to:

- Create a model that is significantly different than the logistic model to predict the height of the CO$_2$ column generated from the growing yeast population.
- Calibrate the model (estimate the parameters) using the collected data.
- Calculate BIC for both the logistic model and the students' alternative model.

We ask students (or student groups) to produce a short written report or present their findings via PowerPoint/Beamer. The reports should include:

- Their alternative model, with a mechanistic explanation for terms.
- Description of solutions and solution procedure, as well as how solution curves do/do not reflect observations.
- Description of parameters required (and their units) as well as estimation procedures.
- A graphical comparison of the logistic and alternate models, along with the data.
- Answers to the questions:
  - Which model better reflects the data, and why? How does this confirm/invalidate any assumptions that you made for your alternate model?
  - For what else could you use this modeling approach?
  - What did you learn from this experience?

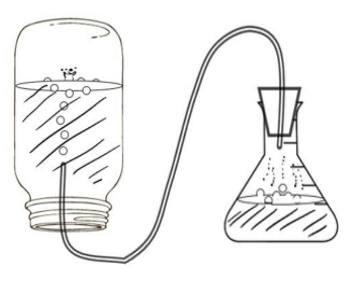
Lab Agenda: Loosely, the in-class portion of the Yeast Lab requires:

1. Lecture: Yeast Lab introduction and data collection setup [15 min]
2. Lecture: Derivation of logistic model [20 min]
3. Group Time: Discussion and development of alternate models [20 min]
4. Lecture: Derivation of BIC and computation of logistic model BIC [15 min]
5. Class Discussion: Groups present alternate models, calibration strategy and BIC score [45 min]

This agenda is typically accomplished over a few class periods, assuming that students are meeting and discussing alternative models and calibration strategies. The details of the schedule can be compressed or expanded as needed (e.g., setup can occur before class). If the lab is used as an example of a mathematical technique (e.g., separation of variables or numerical solutions to ODEs) one of the models discussed below can be provided along with data collected in/before class.

Yeast Lab

Laboratory Experiences in Mathematical Biology



Lab Setup: Yeast is grown in a small, capped flask, generating carbon dioxide which is measured in an inverted jar.

Materials: The following materials are needed (for each group/individual):

- Approximately 500 ml flask, with cork and flexible tube.
- 1 quart Mason jar (or similar).
- 1 plastic container, maybe 1-2 inches tall, large enough to comfortably contain the Mason jar with an inch or two of clearance around the edge of the top.
- iPad with charger and app for time-lapse pictures.
- Food coloring.
- Ruler approximately as tall as Mason jar, two rubber bands. Make sure the ruler and its markings make a nice contrast to the food coloring.
- 1 package dry yeast (1.5 tsp or 4 gm).
- 1/2 tsp granulated sugar (3 gm).
- 300 ml distilled water (room temperature).

Methods:

1. Put 300 ml of distilled water in flask and inoculate with 1/2 tsp of dry yeast. Swirl to mix and allow the yeast to hydrate for 5-10 minutes.

Figure 1: As an alternative to measuring with teaspoons, one may measure the yeast with a scale as seen here

2. While this is happening, organize the visualization apparatus:
   a) Fill the Mason jar to brim with tap water and add a few drops of food coloring for visualization purposes (Figure 2).

Figure 2: Food coloring is added to the water to help with seeing the height of the water when viewing the recording.

   b) Cover with plastic container and with both hands flip over so that the Mason jar is inverted and full of fluid (Figure 3).

Figure 3: When flipping the container be sure to apply a fair amount of pressure on the plastic container to eliminate spillage.

   c) Add a little more colored liquid (around 1 cm deep) to help maintain a seal (Figure 4).

Figure 4: After inverting the jar add additional water (about 1 cm) to help maintain the seal.

   d) Affix a ruler to the side of the Mason jar with the rubber bands.
   e) Set up the iPad application to take a picture every 15 minutes, and then place the iPad (plugged in so it won't run out of juice) to get a good view of the ruler on the side of the jar. Make sure the area will be lighted during the next 24 hours (either leave the room light on or place a small desk lamp nearby to illuminate).

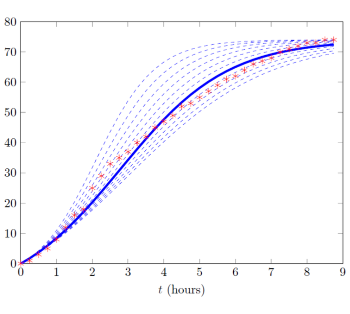
3. Add the 1/2 tsp sugar, swirl again, cap the flask using a stopper with surgical tubing already attached.
4. After swirling has settled down and yeast has begun to bubble ($\sim$ 5 min) snake the surgical tubing underneath the lip of the Mason jar, being careful not to lose the seal on the liquid. Rest the jar back down on the tubing (it will no longer sit perfectly straight -- you can fix this by slipping short, cut pieces of tubing underneath the edges of the jar). You may want to release enough liquid from the jar so that the fluid height is at a uniform cross-section of the jar.

\item Turn on the iPad app and take some data (Figure 5)!

Figure 5: Snake the tubing beneath the jar and position the recording apparatus where the height can easily be recorded. Cap the flask and ensure there will be good lighting for the next 10 hours or so.

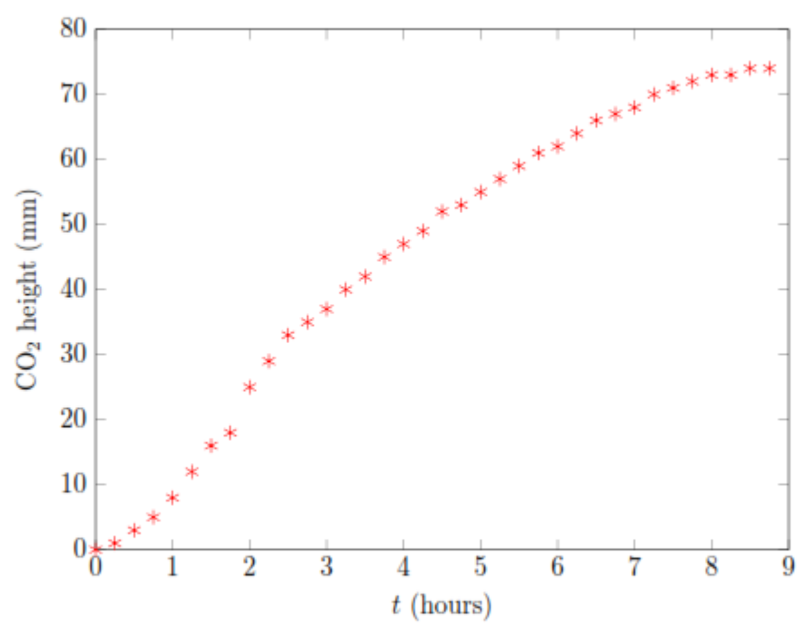
Yeast Lab

Laboratory Experiences in Mathematical Biology



Data and Examples: Data along with some student approaches are presented to illustrate the range of student creativity and to help prepare teachers to scaffold student thinking.

Sample Data: Measured heights of CO$_2$ produced by yeast growing on sugar in a flask. Data was collected by time-lapse video on an iPad, with frames taken every 15 minutes.



$j=$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
$h_j=$	0	1	3	5	8	12	16	18	25	29	33	35	37	40	42	45	47	49
$j=$	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
$h_j=$	52	53	55	57	59	61	62	64	66	67	68	70	71	72	73	73	74	74

Examples of Student Models: Below are examples generated by upper-class undergraduates and graduate students from mathematics, statistics, biology and natural resources in the Fall 2015 Math Biology Lab at Utah State University.

Waste Model: Students focused on the effects of ``waste'' produced during metabolism. Instead of producing only additional yeast cells, as yeast consume and utilize the sugar also contributes to ethanol production during anaerobic respiration, as well as decreasing the pH level as CO_2 bubbles through the solution, making the environment less suitable for yeast reproduction.

The group started with equations for yeast and sugar concentrations as well as the amount of CO_2 produced,

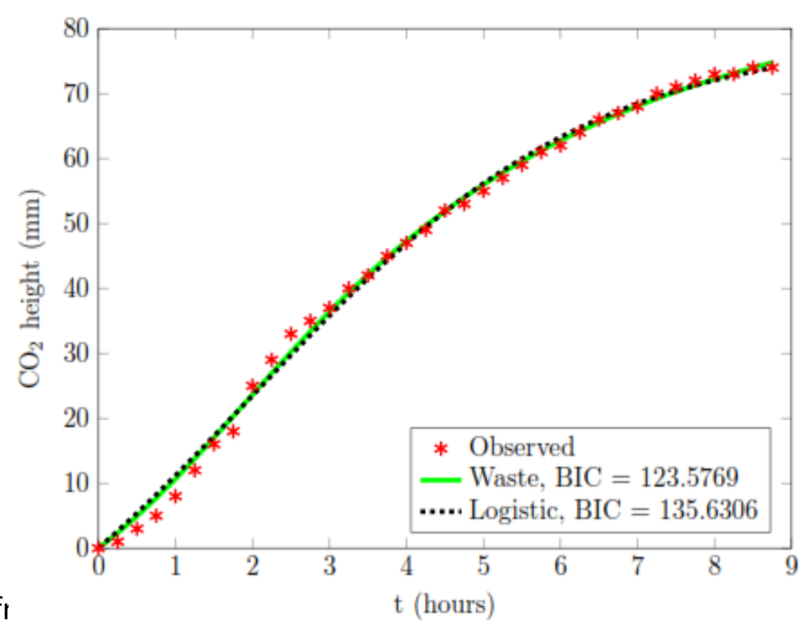
$Y= bYS$

$S =-abYS$

$C = cbYS.$

To capture how waste would affect yeast growth rate, b, the group proposed a "backwards Holling III'" equation of the form:

$b(W)=r_0 W_0 / W_0^2 + W^2 = r_0 W_0 / W_0^2 + (S_0 - S(t))^2$.



Predicted heights fr ; (*) produced by yeast growing on sugar in a flask and the initial logistic model derived in class. Parameters $r_0 = 0.35$, $W_0 = 2.47$, c=25.53 were found using nonlinear least squares and a was set equal to 1.

While the students felt they did well, they specifically mentioned how they were frustrated with the model's performance over the first seven or so data points. However, they could not discover a mechanistic means to ``bend the curve lower'' at the beginning that did not destroy the good fit over the remaining points.

Pyruvate Model: Students leaned more heavily on their biological background. They focused on the differences between aerobic and anaerobic respiration, believing that the growth chamber started out with oxygen in solution which would be used by yeast for more efficient growth until it was depleted. In each process glycolysis breaks down sugar molecules into pyruvate molecules. Once the cell has pyruvate the yeast must continue respiration in either an aerobic or anaerobic direction; this choice is based the presence of O_2. A cell that can perform aerobic respiration will continue on to the aerobic cycle in the mitochondria. If oxygen is not available the yeast will move into anaerobic respiration (fermentation), which is much less efficient and produces alcohol.

Pulling these ideas together, the students wrote

$S=-kYS$

$Y=-kYS+\alpha_o O_2 / O_2 + C P + \alpha_a C / O_2 + C P$

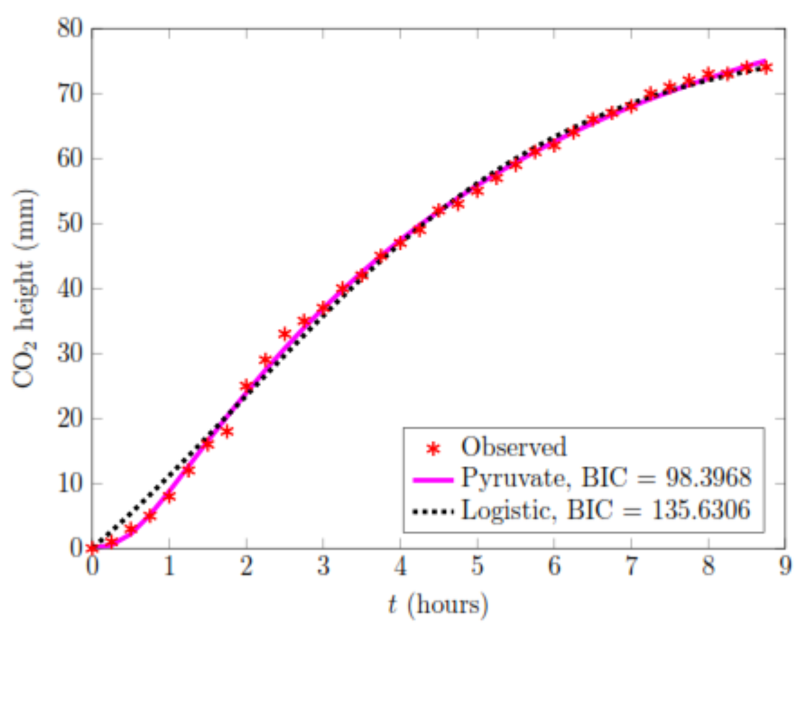
$P=kYS - r_0 O_2 / O_2 + C P - r_a C / O\_2 + C P.$

The students also built equations for the changing levels of O_2 and CO_2.

$O_2 = -\beta_o O_2 / O_2 + C P$

$C = 6\beta_o O_2 / O_2 + C P + 2 \beta_a C / O_2 + C P$

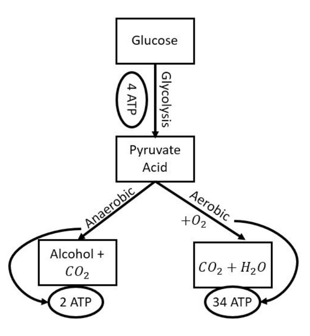
where β_o represents the rate at which O_2 is consumed in the aerobic respiration pathway and β_a is the rate CO_2 is produced in the anaerobic respiration pathway.



Predicted heights from the P uced by yeast growing on sugar in a flask and the initia fminsearch in MATLAB.

Yeast Lab

Laboratory Experiences in Mathematical Biology



Background and Extensions: To build biological context and facilitate in lab presentation, a brief history of yeast in mathematics is discussed along with a short description of cellular respiration.

Why Yeast?

Yeast is one of the oldest domesticated organisms and has a long biological history. Yeast is popular due to its practical importance in the food and beverage industry; as a eukaryotic organism it shares many cellular characteristics and mechanisms with more complicated multicellular eukaryotes, like humans. Pragmatically, yeast works as a model experimental organism because it grows readily on simple media, reproduces by budding and gives investigators absolute control over its environmental parameters. Notably, Gause relied on yeast in *The Struggle for Existence*, a classic in quantitative population ecology. Yeast was one of the first organisms to which molecular approaches were applied to in the 1950's, and in 1996 yeast was the first eukaryote to have its genome sequenced. Ultimately yeast represents ``an ideal system to investigate cell architecture and fundamental cellular mechanisms'' and thus, yeast has been a common experimental choice.

Of the many similarities yeast share with other eukaryotes, cellular respiration is particularly important. Producing energy for most organisms is all about creating adenosine triphosphate (ATP) from sugar molecules. ATP is the basic ``molecular unit of currency'' of intracellular energy transfer, providing energy for most cellular functions, including synthesis of proteins and assembly/disassembly of cellular structures. In the presence of oxygen, the oxidation (burning) of glucose gives

$$\underbrace{C_6H_{12}O_6}_{Glucose} + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

and during this process up to 38 ATP are created (although practically speaking the yield is more like 30 ATP). In the absence of oxygen, however, fermentation occurs and

$$\underbrace{C_6H_{12}O_6}_{Glucose} \rightarrow 2\,CO_2 + 2\underbrace{C_2H_5OH}_{Ethanol},$$

which produces only 2 ATP -- about 15 times less efficient than the aerobic pathway. Presumably the energy generated keeps the yeast cell alive and allows it to bud (reproduce -- yeast cells make little buds, which fall off and become adult yeast cells). As alcohol (ethanol) is produced, fermentation declines and yeast becomes dormant. To generate higher concentrations of alcohol (above 14%) either alternative species of yeast or distillation is required.

Yeast Lab

Laboratory Experiences in Mathematical Biology

Assessment Items: Primary assessment of student learning is taken from students' written reports additional assessment items targeting lab objectives are included here.

Simple knowledge: State the logistic model.

Algorithmic Skill: Calculate the steady states for the logistic equation and determine which are stable/unstable.

Comprehension and Communication: Give a biological interpretation of the steady states of the logistic model.

Comprehension and Communication: Explain what feature in the logistic equation causes the population growth to slow?

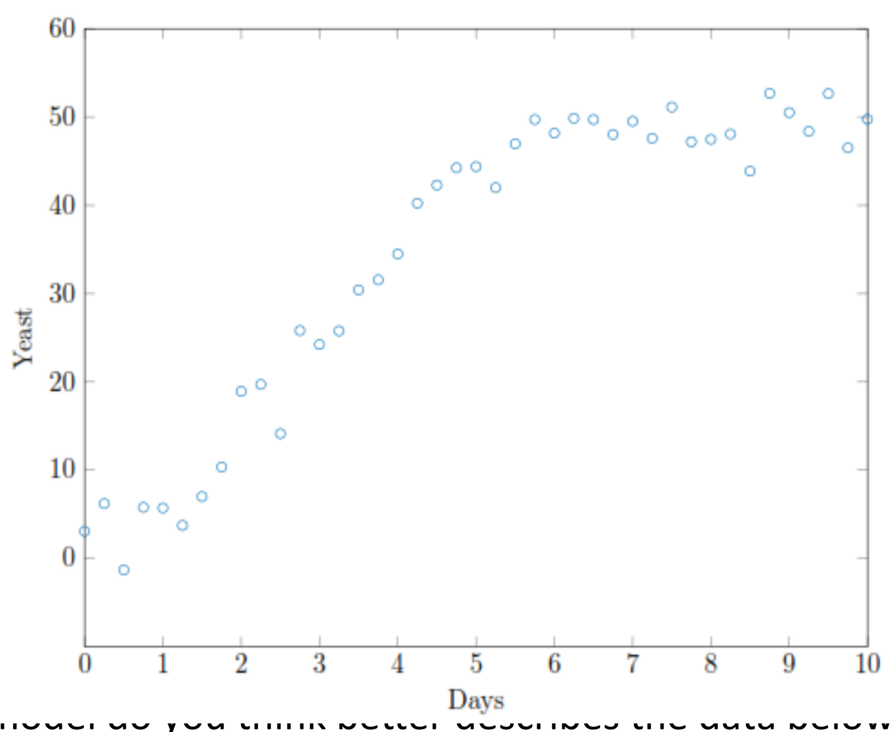
Comprehension and Communication: Below is a proposed yeast model where S is sugar, Y is yeast, and O is oxygen. From this model, make a schematic diagram.

$dS/dt = -\beta YS/N$

$dY/dt = \beta YS/N - \alpha Y$

$dO/dt = \alpha Y$

Discover a Relationship: Explain how the assumptions from the logistic model could relate to the given data (Does the data `look' logistic? What is the carrying capacity and growth rate?)



Application: Which model do you think better describes the data below? Explain why.

$Y = rY(1 - Y/K)$

$Y = rY\ln(K/Y)$