Diffusion

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Biography

Charlotte Omoto is a professor of Biology at Washington State University where she has been since 1984. She received her B.S. in Biology from the University of Washington, Seattle and her Ph.D. from University of Wisconsin, Madison. She has done post-doctoral work at Princeton, Caltech and Penn. State University. Her research interest is focused on protozoan parasites called gregarines. She teaches introductory biology course and a genetics course for non-science majors.

Deborah Short has been the instructional laboratory supervisor responsible for laboratories for the general biology course for non-majors, introductory biology course for majors and general ecology course for many years. She received her B.S. in Biology from Washington State University.

Paul Rabie is a Ph. D. candidate and has been a teaching assistant in introductory biology and ecology courses for several years. He received his BA in Fiction Writing from Hampshire College (Amherst, MA) and MS from Univ. of Minnesota in Ecology. His research interest has been themed around the problem of invasive species. Currently he is developing methods to rigorously evaluate relationship between individual physiological performance and population dynamics to be useful to practitioners evaluating the potential for introduced species to become invasive.

Introduction

This laboratory exercise is suitable for introductory level majors biology course. The set-up is quite simple and can be done ahead of time. The exercise as presented does not take a full 3-hour lab period. We have done a variety of different related activities such as measurement of heat loss from hot water in different sized beakers (be sure to heat the beakers with hot water then add more fresh hot water). Bob Kosinki's lab on Osmosis presented earlier in this volume would also be a good complement to this laboratory exercise.

Background on diffusion

Diffusion is the random movement of molecules due to thermal energy. The Einstein diffusion equation can be derived as follows:
Kinetic energy of molecules due to movement = $mv^2/2$ where m is mass and v is velocity. Kinetic energy due to thermal energy = kT/2 where k is the Boltzmann's constant and T is absolute temperature. Therefore $\frac{kT}{2} = \frac{mv^2}{2}$. Rearranging, we get $\sqrt{v^2} = \sqrt{\frac{kT}{m}}$.
This equation allows us to calculate the velocity for a molecule given its mass and temperature. For oxygen, the velocity is ~250 m/sec; at that rate, it can cross a football field in ~0.5 sec. But does it? No, because it runs into other molecules!

One analogy is the collegiate football game when fans thought the game was over and ran out onto the field when the game was actually not over. The football player with the ball had to dodge fans and marching band members to try to make a touch down. It took him a lot longer to cross the football field than when he returned a kick-off return with hardly anyone on the field.

We cannot speak of diffusion as rate because farther molecules spread, it takes much more than proportional amount of time. Diffusion coefficient is a measure of how long it takes molecules to diffuse. The unit for diffusion coefficient is distance^2/time, rather than distance/time, which is the unit for rate or velocity. The implication of distance^2 in the diffusion coefficient is that for a molecule to diffuse double the distance, it takes 2^2 or 4 times the time, and for it to diffuse 10 times the distance, it takes 10^2 or 100 times the time. The equation for a straight line is y = mx + b. By taking log of each side gives: log of time = 2 log of dist. Thus plotting on a log-log graph should produce a straight line with a slope of 2.

In the case of oxygen in water, it takes only about 10 msec to diffuse 6 μm (about the size of our cells), it takes about a whole second to diffuse 60 μm (size of a typical protozoa), about 3 hours to travel 6 mm and over 24 hours to diffuse 2 cm. Of course, in a protozoan such as paramecium, oxygen and other molecules does not rely solely upon diffusion, but use cytoplasmic movement to facilitate transport of molecules.

Diffusion is a *very efficient* way to transport molecules over short intracellular distances, but is totally inadequate for transporting molecules even over mm to cm distances. The lab exercise involving the plastic cubes can thus be extended to discussions of increasing the surface area to enhance diffusion of gasses and nutrients. This then can be related to biological structures that increase the surface area, such as gills and intestinal epithelium.

Student Outline

Introduction

Most single-celled organisms are microscopic; only the largest of them are visible to the naked eye. The cells that make up multicellular organisms are also microscopically small. Very large cells are rare and are made for special circumstances (such as an egg of an ostrich). So why are most cells small? The answer lies in how cells interact with their environment. Everything that enters or leaves a cell must do so through the cell surface, most often through diffusion. Typically, as the volume of a cell increases, the surface area per unit volume decreases. A large cell simply could not bring in materials and get rid of waste fast enough because of its limited surface area relative to its volume. Some multi-cellular organisms, such as flat worms, absorb nutrients directly through their entire body surface. Their flattened shape maximizes the surface area to volume relationship. Larger, more complex animals must rely on adaptations such as circulatory systems and specialized organs for gas exchange, nutrient absorption, and waste removal.

In the first exercise, we will measure diffusion into different sizes of "cells" represented by blocks of agar. The agar has been dyed with a pH indicator that is pink at slightly basic pH but loses color in neutral and slightly acidic conditions. While the experiment is going on, we will explore the surface area to volume relationship using plastic cubes. Finally, we will analyze the diffusion process by graphing.

Exercise 1a - Surface Area to Volume Relationships, Diffusion

Materials

Agar blocks made with phenolphthalein indicator
Ruler
Non-serrated knife
Vinegar
White paper towels
Soaking pan
Graph paper
Calculators

Procedure

1. Invert the container with the colored agar to release the block of agar onto a paper towel. Trim the block so that the sides are straight up and down rather than sloping. Measure and cut blocks with the following dimensions:
    a. One block 4 x 4 x 4 cm
    b. One block 3 x 3 x 3 cm
    c. **Two** blocks 2 x 2 x 2 cm
    d. One block 1.5 x 1.5 x 1.5 cm

e. One block 1 x 2 x 2 cm
f. One block 1 x 1 x 1 cm

We suggest that you cut the largest block first. You should have enough agar for all the blocks. Return unused pink agar into a bucket in the front of the class.

2. Arrange the cut blocks in the container so that they do not overlap. Pour in enough vinegar to float the blocks. Start the timer.
3. Allow the blocks to soak in the acidic vinegar just until the smallest cube loses all of its pink color. While you wait for this to happen, do Exercise 1b.
4. When the smallest cube has lost all of its pink color, note the **time and immediately** remove all of the blocks except 1.5 x 1.5 x 1.5 cm and one of the 2 x 2 x 2 cm from the vinegar and put them on a paper towel. Keep an eye on these two, and note how long it took each of them to lose all of its pink color. This may be easier if you put the container on white paper. Note the time in the table below, and use the information in **Exercise 1c**.

Table 1. Time for blocks to clear

Block size in cm	Distance diffused in cm	Time to clear in min.	"Rate" of diffusion in cm/min
1 x 1 x 1			
1.5 x 1.5 x 1.5			
2 x 2 x 2			

5. Without waiting a long time, measure the distance that the vinegar diffused from each of the 6 planes for each block and average the distance for each block and enter in the following table.
6. Measure the dimensions of the pink area within all blocks that still have a pink center. Calculate the volume of the pink area in each block (Volume = length * width * height). Enter these volumes in the table below.
7. Calculate the volume into which the vinegar diffused for each block (diffused volume = total volume – pink volume). Enter this volume into the table below.
8. Calculate the percent of the total volume into which the vinegar diffused and enter it in the table below. (Percent diffused = 100 * diffused volume / total volume).
9. Graphing the data from an experiment gives us a better picture of the surface area to volume relationships we explored. In a typical graph, the independent variable–the variable determined before doing the experiment–is plotted on the X-axis. The dependent variable–the variable or value measured in the experiment–is plotted on the Y-axis. On a graph, plot the surface area of the blocks (the independent variable) on the X-axis in relation to the diffused volume (the dependent variable) on the Y-axis.

Table 2. Data on distance diffused

	Block 1 1x1x1 cm	Block 2 2x2x2 cm	Block 3 3x3x3 cm	Block 4 4x4x4 cm	Block 5 1x2x2 cm
Surface area of each block					
Total Volume of each block					
Surface area to volume ratio					
Average distance diffused					
Pink volume remaining after experiment					
Diffused (colorless) Volume					
% Diffused Volume					

Questions:

1. What do the agar blocks represent? What might the diffused volume represent? What implications might this have for "cells" with different % of diffused volume?
2. In addition to the smallest cube, did any of the other blocks lose all their color? If so, explain why.
3. What relationship, if any, does the distance diffused have with either surface area or volume?
4. What relationship does the diffused volume have with surface area?

Exercise 1b - Surface Area to Volume Relationship with Blocks

While you wait for the vinegar to diffuse into the agar blocks, explore surface area to volume relationships using plastic cubes and fill out the first three rows of Table 2 above. Each group will get 27 plastic cubes with dimensions of 1 cm^3.

1. Examine a single cube. What are the surface area, volume, and the surface area to volume ratio? (For this exercise, ignore the surface area of the indentations and knobs on the blocks.)
2. Make a cube that is 2 cm on each side. What are the surface area, volume, and surface area to volume ratio for this cube?
3. Make a cube that is 3 cm on each side. What are the surface area, volume, and surface area to volume ratio?
4. Compare the figures for the three cubes. What is the relationship of the increase in surface area to increases in linear dimension (the length of one side of the cube)? What is the relationship of the increase in volume to increases in linear dimensions? Compare the

surface to volume ratios you calculated in the progressively larger cubes.

5. If the three cubes you constructed represent cells, how might the sizes and the surface area to volume ratio affect their functioning?
6. Using 27 small cubes, build a structure that has the *lowest possible* surface area to volume relationship.
   What is the surface area? _____
   What is the volume? _____
   What is the surface area to volume ratio? _____
7. Using 27 small cubes, produce a structure that has the *largest possible* surface area to volume relationship.
   What is the surface area of the structure? _____
   What is the volume of the structure? _____
   What is the surface area to volume ratio? _____
8. Using 27 small cubes, produce a structure that has a large surface area but that is at most 5 cm long in any one dimension.
   What is the surface area of the structure? _____
   What is the volume of the structure? _____
   What is the surface area to volume ratio? _____

Which of the shapes you produced in steps 6-8 do you think would be most efficient in conserving heat? Why?

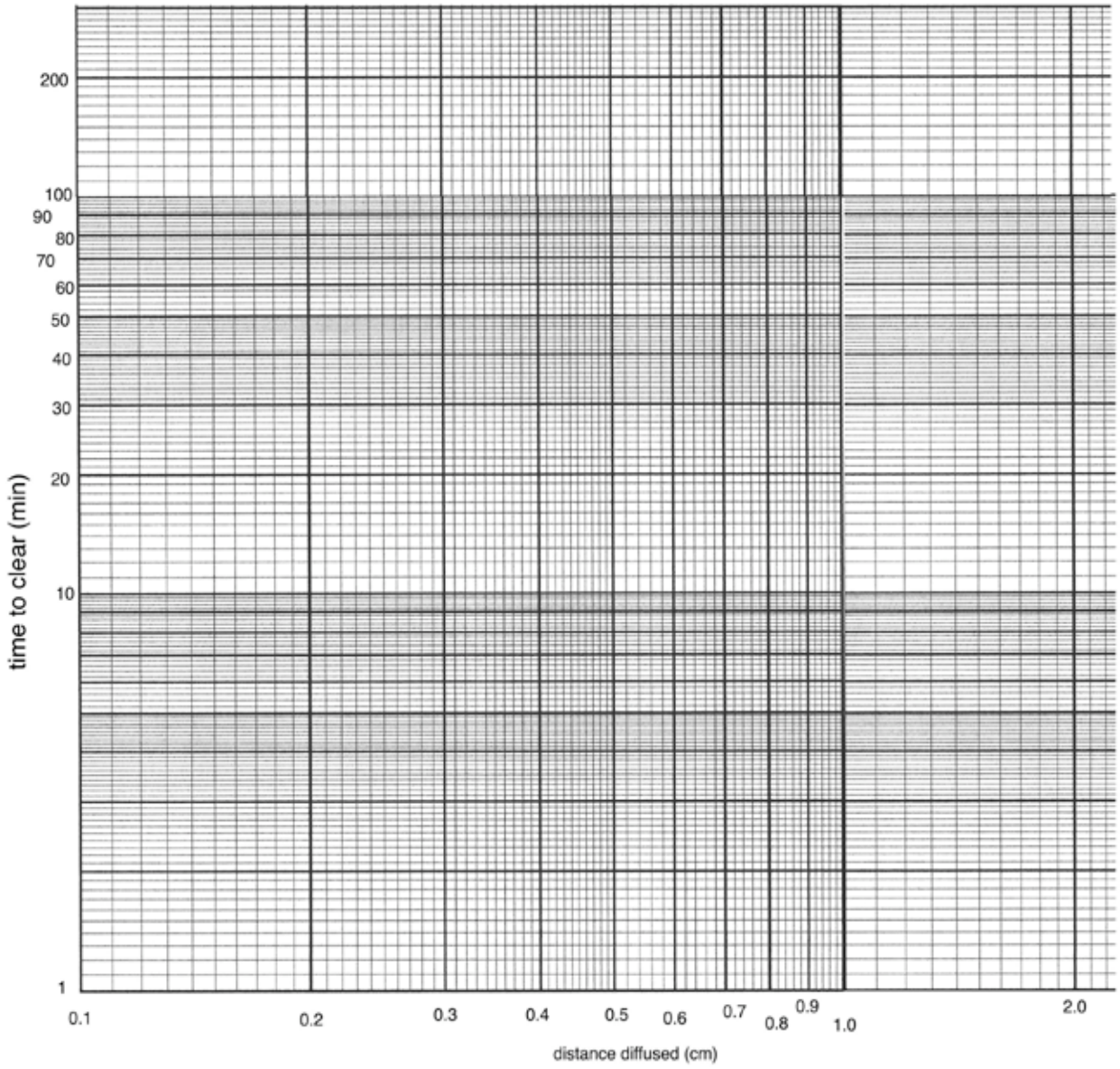
Exercise 1c – Time to diffuse

1. Graphing the data from our measurement of time to diffuse graphically shows the nature of diffusion. Since acid needs to diffuse only half the dimension of the cube, divide the linear dimension in half in the table. Now plot the distance diffused, the independent variable on the X-axis and time in minutes to clear, the dependent variable, on the Y-axis.
2. We expect this to be a power function (and what do you expect the power to be?) A power function plotted on a log-log plot will give a straight line. Try plotting the same data on a log-log plot (at the end of the lab exercise. Do the points fall closer to a straight line?

Questions:

1. Can you estimate how long it would take for a block that is 0.5 x 0.5 x 0.5 cm to lose all its pink color? What about the largest block of 4 x 4 x 4 cm?
2. Approximately how much longer does it take for the vinegar to diffuse double the distance from 0.5 cm to 1cm?
3. If you wish, cut a 0.5 x 0.5 x 0.5 cm block to check your prediction.
4. In calculating the "rate" of diffusion for 1 x 1 x 1 cm block, 2 x 2 x 2 cm block, why is the "rate" of diffusion different?
5. Can you see now why it is misleading to use the term "rate of diffusion"?

Log-Log graph for Exercise 1c



Notes for the Instructor

For a lab section of 24 students divided into 6 groups
Each section needs 4 tubs of agar: 1/2 tub per table plus one extra for mistakes.
Each tub holds about 625 mL (Ziploc brand, square shape, 20 oz.)

Agar Block recipe For 8 tubs:
In a large kettle,
Mix: 5 L deionized water (can be slightly generous in measuring)
50 g agar
Autoclave for 3 minutes to melt agar completely
Add: 50 mL phenolphthalein
2 mL 10% NaOH* *(for smaller batches - 1 mL = 20 drops)
Mixture should be quite pink
Pour into plastic tubs – fill to the ledge just below the rim
(Must be at least 4 cm deep for the exercise).
When solid, cover surface of agar with plastic wrap, put on lid, store in the refrigerator.
This can be made several days ahead if kept in the refrigerator and surface sealed from air.

Phenolphthalein recipe (Phenolphthalein is a powerful laxative – avoid inhaling powder)
For 100 mL
Dissolve 0.05 g phenolphthalein powder in 50 mL 95% ethanol
Add 50 mL H_2O

Place beaker over white paper to be able to see color easily
Add 0.4% NaOH drop by drop until just a very slight pink color develops

To unmold the blocks, slide a knife along the edge to break the seal and invert onto a paper towel and cut each block in half. Cut the largest cubes first, starting at one end of the agar block. Be sure to remove the slanted and curved surfaces of agar that were in contact with the tub.

On student tables: non-serrated knives, rulers, timers, a plastic tub for soaking the blocks in vinegar, graph paper, and calculators

For multiple lab sections:
Have students put the used and extra agar in the collection bucket at the side bench and pour the used vinegar back into the jug (use funnel provided). The vinegar easily lasts 2-3 lab sections.
Unused pink agar blocks can simply be melted in a microwave oven and combined into the mold and allowed to gel.
You can reuse used agar blocks by melting it in a microwave oven then using base to adjust the pH so that the pink color is regenerated before remolding.

There are a number of websites that allows you to generate exactly the graph paper you need. One such website is http://incompetech.com/graphpaper/logarithmic/. One can also generate logarithmic graph paper in spreadsheet software such as Microsoft Excel.

We purchased our plastic cubes from EAI Education
http://www.eaieducation.com/531062.html
Some workshop participants suggested using the same mass of plastic play dough. It would be difficult to calculate the surface area, but would allow for greater diversity in shapes

Exercise 1a Some students do not make the connection between the agar blocks and biology. Thus we have questions in section a to specifically ask what the agar and diffused volume represents.

Exercise 1b The plastic blocks allow student with less math skills to determine the surface area and volume simply by counting. The structures that students come up with for 8 can be the starting point to discussing similar biological structures and why those structures must have a large surface area. The last question in Exercise 1b compels students to relate this activity to another relevant biological question of heat exchange.

Exercise 1c The distance diffused on the first table in Exercise 1a is half the linear dimension since the vinegar needs to only diffuse half the distance from all sides for the blocks to clear. By calculating the "rate" and they see that the rate is different for each of the 3 blocks.

Once they plot their data on the log-log graph, they should be able to draw a straight line through all 3 points with a slope of ~2. The "origin" of a log-log graph is arbitrary and the line does not need to go through this "origin". By extrapolating in both directions, they can estimate how long it would take for a smaller block or the largest block we used to clear. They can see how quickly a 0.5 x 0.5 x 0.5 cm block will clear and they can check their prediction.