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Tuesday Lab

9/26/23

Laboratory 2- Molecular Activity and Membrane Transport

Purpose: To investigate the basic properties of passive transport including diffusion, osmosis, and differential permeability.

Procedures:

2-B: Measurement of diffusion through a liquid

Procedure

1. Working in groups, fill three Petri dishes with 40 ml. of 25 C water.

2. Drop one crystal of potassium permanganate into each dish. Be sure to use the same amount of potassium permanganate for each dish. Record the time.

3. Measure in millimeters and record the largest diameter of the colored spot after 5 minutes.

4. Repeat steps 1-3 for water at 5 C and at 45 C.

5. Construct a graph of ranges and means for each temperature.

6. Based on your knowledge of diffusion, what is an explanation for these results?

2-C: Measurement of diffusion through agar

Procedure

1. Petri dishes have been filled with agar. Two holes have been made in the agar. Into one hole, place two drops of methylene blue. Into the other hole, place two drops of potassium

permanganate. Record the time and immediate diameter of each spot. This will be your time zero measurement.

2. Measure the diameter of each spot in millimeters once every minute for fifteen minutes. Calculate the averages from the data collected by all groups doing this exercise. Summarize this data.

3. Construct a graph of average diffusion diameter versus time for both chemicals.

4. Determine the diffusion rate of each chemical. Which has the fastest diffusion rate, methylene blue or potassium permanganate? Record these results.

5. Look up the molecular formula and structure of methylene blue and potassium permanganate in a Merck Index. Make note of this information.

6. Interpret your result with respect to the information obtained from the Merck Index.

2-D: Demonstration of filtration

Procedure

1. Fold three filter papers into cones and insert them into three separate glass funnels. Wet the paper to make them stick to the glass.

2. Prepare three 100-milliliter solutions of charcoal and water. Make one thick, one medium thickness, and one thin. Record the mass of the charcoal used in each preparation. NOTE: if your "thin" solution continually runs through the filter, making it impossible to count drops, it is too thin; you will need to make all your solutions proportionally thicker.

3. Pour 50 ml (about 1.69 oz) of each solution, one at a time, into a funnel.

4. Immediately count the number of drops produced per minute. NOTE: it may be easier to count the drops for 15 seconds then multiply by four to obtain drops per minute.

5. Count the number of drops per minute when the funnel is half-filled.

6. Count the number of drops per minute when the funnel is nearly empty.

7. Did the charcoal pass into the filtrate? Which solution had the fastest rate of filtration? What is the driving force behind filtration? What other factors influence the rate of filtration? Do your results illustrate these influencing factors?

8. Repeat these procedures with the remaining 50 ml of solution.

2-F: Measurement of osmosis

Procedure

1. Attach dialysis bags filled as much as possible with sucrose solutions securely to the bottom of two open, thin glass tubes. One bag should be filled with a 25% sucrose solution and the other should be filled with a 50% sucrose solution. Make sure the ends of the tubes are immersed in the solutions. NOTE: reliable results depend on your ability to tightly seal the dialysis bags.
2. Insert both bags into separate beakers of distilled water making sure the dialysis bags are fully submersed but not touching the bottom of the beakers and suspend each by gently applying a ring stand clamp to the glass tubes. Check for solution leaking out of the bags.
3. Allow five minutes for the systems to equilibrate. Then, mark the fluid levels of each glass tube with a felt pen. Record the time.
4. Record the fluid level of the glass tubes in millimeters every 10 minutes for 50 minutes.
5. If the fluid level rises to the top of the glass tube sooner than 50 minutes, record the time it took to get there, measure the length in millimeters from the equilibration line to the top of glass tube. Divide that length by the number of minutes to get your rate in mm/min.

6. Determine the rate of osmosis for each system. Which system had the fastest osmotic rate, the 25% or 50% sucrose solution? Explain these results.

2-G: Measurement of differential permeability of sugar and starch

NOTE: In this experiment, chemical indicators will be used to determine the presence of starch and sugar. Lugol's solution, an amber iodine-containing reagent, will turn dark navy blue in the presence of starch. Benedict's solution, a blue cupric (Cu+2) solution, when heated in the presence of a reducing sugar, will be reduced to form a reddish precipitate of cuprous oxide (Cu2O). The Benedict's solution will change different colors, ranging from green to red, depending upon the amount of sugar present.

Procedure

1. Fill a dialysis bag with a 1% starch –10% glucose solution. Reliable results depend on your ability to tightly seal the dialysis bag.

2. Tie the bag to a glass rod and suspend it in a beaker of distilled water. NOTE: Test the water from the bottom of the beaker to ensure that it is free of starch and/or sugar.

3. After 15 minutes has passed check the water again for starch and sugar in the following way:

Test for starch:

a. Add 10 drops of Lugol's solution to 5 ml of water obtained from the beaker.

Reddish color = No starch

Navy blue color = Starch present

Test for sugar:

a. Add 3 ml of Benedict's solution to 5 ml of water obtained from the beaker. Simmer the solution at a low boil for 5 minutes.

Blue color = No sugar

Color change = Sugar present (green = little sugar; yellow = moderate sugar;

orange = more sugar; red = lots of sugar)

4. Test the water in the beaker again at 30, 45 and 60 minutes.

5. Record these results. Explain the significance of these findings in relation to the permeability of the dialysis bag.

2-H: The effects of tonicity on red blood cells -Demonstration

Tonicity refers to the solute concentration of solutions. Hypertonic solutions have a higher solute concentration than the cells in this solution. Red blood cells should shrink or crenate due to osmotic loss of water in hypertonic solutions. Hypotonic solutions have a lower solute concentration than cells in this solution. Red blood cells in a hypotonic solution swell and will eventually undergo cytolysis due to osmotic gain of water. Isotonic solutions have the same solute concentration as cells in this solution. Red blood cells in isotonic solutions slightly swell and shrink in a dynamic equilibrium with their medium. In this exercise, the effect of hypertonic, isotonic, and hypotonic solutions on red blood cells will be observed.

Procedure

1. One milliliter of each of the following solutions will be in three separate test tubes.

a. Distilled water (hypotonic)

b. Physiological saline –0.85% NaCl (isotonic)

c. Salt water –2.0% NaCl (hypertonic)

2. A small drop of blood will be added to each tube and the contents thoroughly mixed.

3. A wet mount slide will be made of each solution.

4. Examine each slide under the high-dry lens of a compound microscope.

5. Observe the following:

a. Hemolysis of cells in the hypotonic solution. (Note the transparent solution.)

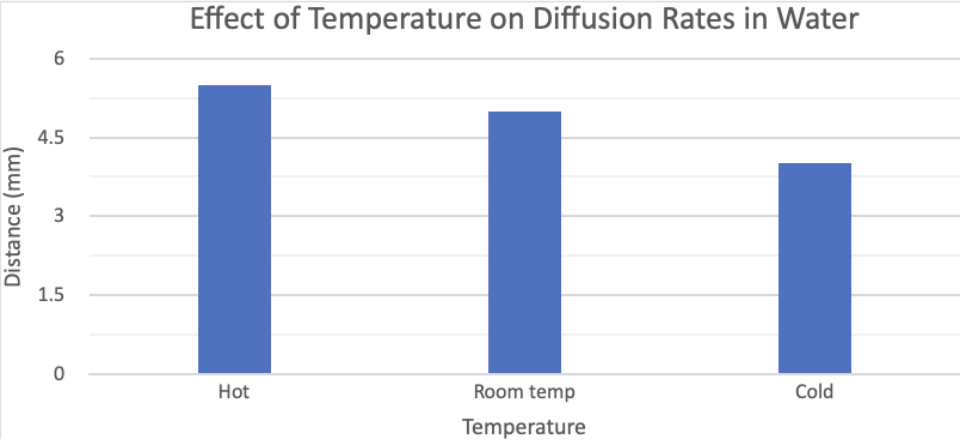
b. Maintenance of cell size in the isotonic solution.

c. Crenation of cells in the hypertonic solution.

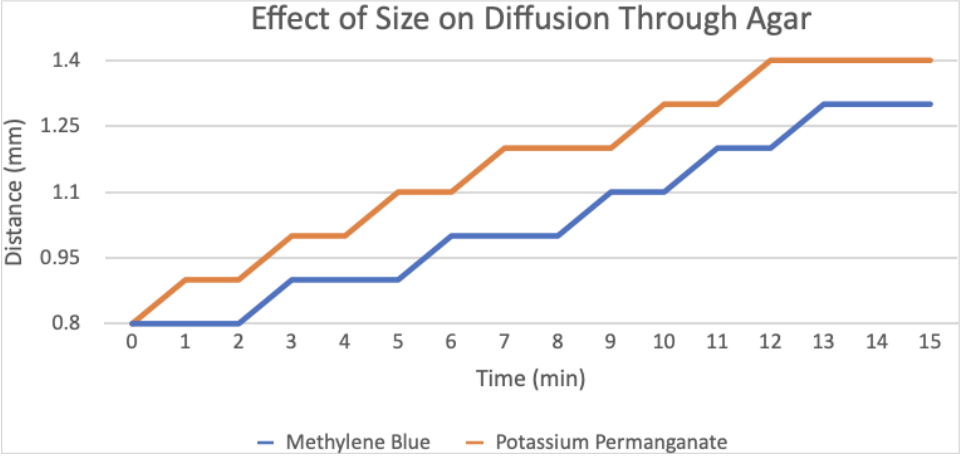
6. Make a drawing of each observation and provide an explanation for each.

Results:

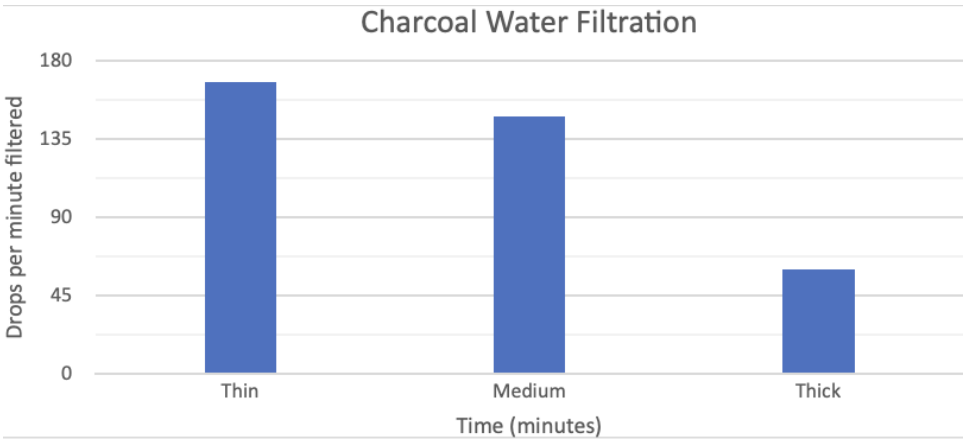
2-B



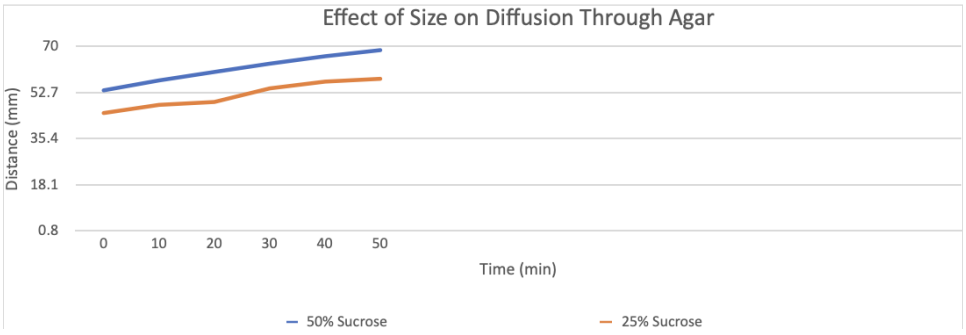
2-C



2-D



2-F



2-G

Time (min)	A (Starch)	B (Sugar)
15	**none**	**Little sugar**
30	**none**	**Moderate sugar**
45	**none**	**More sugar**
60	**none**	**Lots of sugar**

Discussion: During all these labs I got to see that diffusion, filtration, and osmosis are all processes that involve the movement of substances. Diffusion is the movement of particles from an area of high concentration to an area of low concentration. Filtration is the process of separating solids from liquids or gases by passing them through a filter. Osmosis is the movement of water molecules across a semipermeable membrane from an area of low solute

concentration to an area of high solute concentration. These processes are important for maintaining balance and homeostasis in living organisms.

Conclusions: To conclude from my data, I now know that diffusion is temperature and size dependent, filtration is dependent upon solution density, and osmosis is concentration dependent.