The Microbial Lava Lamp - Teachers Guide

(version 2.9)

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This activity can be used as a demonstration or as a hands-on, inquiry-based exercise. Yeast cells are immobilized in beads of alginate. Alginate is a carbohydrate polymer derived from "seaweed" and used in the food and pharmaceutical industries. The beads containing the immobilized yeast cells are placed in a sucrose solution and through yeast metabolism, carbon dioxide gas bubbles will form on the bead surface. The gas bubbles cause the beads to rise to the surface of the sucrose solution. Once the carbon dioxide bubbles are released from the beads, the beads will fall to the bottom of the sucrose solution until they generate sufficient carbon dioxide to rise again.

The same carbon dioxide-forming activity of yeast is used in baking bread. Just as the carbon dioxide is formed through yeast activity in the sucrose solution, carbon dioxide is formed and released in bread dough causing it to rise. Yeasts are also involved in the fermentation of the sugar in fruit juices to make wine and the malt sugar to produce beer. Other microorganisms are involved in other fermentations and the products produced are sometimes used in food production and preservation. The holes in Swiss cheese are caused by large bubbles of carbon dioxide released by a fermenting bacterium. Yogurt, sour creme, cottage cheese, a variety of other cheeses, sourdough bread, sauerkraut and pickles are examples of food products produced through some type of microbial fermentation. Students might be interested to see some packages of these actual food products; this can serve as a good point for student discussion while they observe the CO_2 evolution in the "lava lamp".

Students will be involved in preparing the alginate beads with the immobilized yeast cells and placing the beads in the sucrose solution. The yeast cells will start respiring actively in a few hours, and will continue to do so over several days. This means that the apparatus (the "lava lamp") can be put together one day and observed on subsequent days. The lamps can be stored in a refrigerator for a few days, if necessary, and returned to room temperature to regain activity. Initially the CO_2 produced by the yeast is due to cellular respiration (aerobic respiration). Oxygen in the sucrose solution is consumed during aerobic respiration. Eventually the oxygen level becomes too low to support aerobic respiration and the yeast will start fermenting some of the sugar to ethanol. Some CO_2 will still be produced. As time goes on, an acetic acid (vinegar) smell may be noticed. This is from oxidation of the ethanol to acetic acid by naturally-occurring bacteria present in the apparatus. A similar process is used to make vinegar from wine.

The "Microbial Lava Lamp" activity can be used to introduce the concepts of respiration, enzyme activity, metabolism, biochemistry and microbiology. Since the students create the beads and place them in the sucrose solution, they will get a sense of "ownership" which should stimulate interest. The optional addition of colored pigments to the beads should also stimulate interest in the activity (although see the precautions). The use of a fermentation lock bubbler allows the rate of the enzyme reaction to be monitored by counting the number of bubbles over a given time period. Reaction rates (bubble production) from "lava lamps" exposed to different environmental conditions can be compared.

The activity can also be used to expose students to the idea that immobilized cells (and immobilized enzymes) are sometimes used in biotechnology to carry out chemical or biochemical reactions. In some cases immobilized cells remain alive for longer periods of time and may be more stable to environmental changes than their non-immobilized counterparts. There are a number of other extensions to chemistry, physics, and mathematics.

The "Microbial Lava Lamp" activity lends itself to inquiry or discovery-based learning, the scientific method, and design of scientific experiments. Students can make observations, record and tabulate or plot data, generate hypotheses, and conduct experiments to test their ideas. The activity allows students to work and conduct experiments in groups, but individuals can easily work on their own experiments.

Please review the <u>Precautions</u> section (below) before starting the exercise.

Ingredients Included in Kit:

1. Alginate powder (Keltone LV from Nutrasweet Kelco). Alginate is also known as alginic acid, sodium salt; sodium alginate; or algin. We will refer to it as alginate in this handout. **The Keltone LV alginate supplied with this kit has been generously donated by the Nutrasweet/Kelco Company.**

2. Spheriglass powder glass (C5 size. Potter's Industries)

3. Fluorescent pigments (microencapsulated Glo-Sperse EPX. DayGlo Color Corp.)

4. Calcium chloride ($CaCl_2$)

5. Disposable Gloves for students (if fluorescent pigments are used); 12 universal size)

Consumable ingredients 1-5 are included in the kit. Refill kits are available from the Biology Education Enhancement Program, Department of Biology, California State University, Northridge. Please use the contact information on the front page for availability. The alginate, glass powder, and fluorescent pigments are sold only in bulk quantities by the manufacturers. Purchasing supplies directly from the manufacturer is probably not feasible for most schools. These items are currently available from the Department of Biology, California State University, Northridge. In some cases the manufacturers may be able to provide limited-quantity samples of these products. Sigma Chemical Co. (St. Louis, MO, 800-325-3010) sells alginate in quantities as small as 100 g for a reasonable price. The Sigma catalog number is A 2158 for Alginic Acid, Sodium Salt (low viscosity). The glass powder is available from companies that carry sand-blasting products. Calcium chloride can be purchased from a variety of chemical supply companies and may be available from your school's chemistry department. Disposable gloves can be found at discount warehouses (e.g. Costco, Sam's Club) or food service supply companies, or perhaps at local grocery stores.

Equipment Included in Kit:

- 1 100 ml Graduated Cylinder
- 1 One-Quart Zip Lock Bag for preparing alginate stock solution
- 2 Powder Funnels for adding beads to sucrose solution
- 6 50 ml Mixing Chambers
- 6 Clear Plastic Tumblers or Plastic Glasses for forming beads
- 6 Plastic Transfer Pipettes to add Glo-Sperse and to form beads
- 5 Bubblers (Fermentation Airlocks) and One-Holed Rubber Stoppers - one for each plastic bottle to be used
- 5 Straws (in 1 Qt Zip Lock bag listed above)
- 1 Mylar balloon to collect carbon dioxide gas

(Note: the above items can be cleaned and reused many times for future experiments)

To be provided by teacher:

- Sucrose (table sugar). A 5 -10 lb. bag is convenient. Prepare a 10% solution (100 g per 1000 ml water; 1/2 cup granulated table sugar (sucrose) is approximately 100 g) Make enough solution to fill soda bottles 3/4 of the way.
- "Active Dry" or freeze-dried yeast. "Quick-Rise" or "Fast-Rise" yeast can also be used. Typically sold in grocery stores as a set of three 1/4 oz. (or 7 g.) packets. Production of 5 "lava lamps" will require all three packets. If more yeast is required it might be more economical to purchase it in small 4 oz. jars. Tightly-closed jars of yeast can be stored in a freezer (-20 ^{o}C) for up to 4 months after opening or until the manufacturer's expiration date, whichever comes first.
- 1 to 5 clear plastic bottles (2 liter soda or 1.5 liter water; you can have the students bring these in a few days ahead of time. Smaller water bottles can also be used as long as the rubber stoppers included with the kit will fit tightly. Bottles should be rinsed well with tap water before use.) Sufficient ingredients are included in this kit to make 5 "lava lamps". Ninety mls of alginate solution (e.g. the amount in three mixing chambers) will yield enough beads for one 2 liter or 1.5 liter "lava lamp".
- 1 Thermometer (capable of reading up to 110 ^{o}C). Non-mercury thermometers are preferred in case of accidental breakage.
- Measuring cups (1 cup, ½ cup) and measuring spoons (1 tablespoon, ½ tablespoon, ¼ teaspoon) or a balance capable of weighing 1 to 200 g
- Nylon stocking or a tea strainer for trapping and washing beads.
- Newspaper
- Rubber bands to attach mylar balloons to straws
- Pyrex Glass Beaker 600-1000 ml (to fit in coffee pot below)
- A blender or whisk and mixing bowl for preparing alginate solution (optional)
- A small inexpensive coffee pot or other method to heat alginate solution (in 50 ml mixing chambers) to 41-46 ^{o}C (105 - 115 ^{o}F). A Rival "Hot Pot Express" pot works well.
- Distilled or deionized water. (Ordinary tap water is OK).

Procedure: Encapsulated yeast bead preparation

A. Teacher:

Advance preparation of alginate solution:

Store the alginate powder in a cool, dry location until use. Prepare a 1.8 % - 2% solution (1.8 - 2.0 g per 100 ml water, or 9- 10 g per 500 ml water) before class use. Approximately 9- 10 g of Keltone LV Alginate is supplied with each 20-student kit. There are three ways to prepare the alginate solution:

1. Blender Method - Add the alginate powder to 500 ml of water in a blender. Blend at medium speed for about a minute. Make sure that the powder has dissolved. Repeat if necessary to dissolve the alginate. Heat* the mixture in a boiling water bath for 5 min. Keltone LV is a food grade product and the blender can used for food after it has been cleaned.

2. Whisk/mixing bowl Method - Add the alginate powder to 300 ml of water in a bowl. Rapidly mix the alginate power with a whisk. When most of the alginate clumps have dissolved, add the remaining 200 ml of water and continue mixing until the powder has completely dissolved. Heat* the mixture in a boiling water bath for 5 min.

3. Zip-Lock Bag Method - Place 9 g of alginate powder into a clean 1-quart heavy-duty zip lock bag. Add 100 ml water to the bag, remove as much air as possible and seal the bag. Check the seal. Knead the contents of the bag to disperse the alginate into the water. While laying the bag on a bench or table top, squeeze large alginate clumps with fingers. Continue kneading the contents of the bag for several minutes. After most of the big clumps have been removed add an additional 100 ml of water to the bag. Continue the kneading process for several more minutes. Again, keep working on dispersing all the alginate clumps into suspension. Large clumps will yield inconsistent and undesirable results. When no alginate clumps remain, place the plastic bag upright in a large glass beaker. Open the bag and add 300 more mls of water to the alginate solution. Then surround the bag with a little water. Heat* the mixture in a boiling water bath for 5 min.

***Heat-treating the alginate solution:** You can use a microwave oven and a microwave-safe container or other type of heater (such has a hot-pot). Heating the mixture in a boiling water bath for 5 min. helps to disperse undissolved alginate particles and lower microbial contamination. Extensive microbial contamination might otherwise reduce the shelf life of the alginate solution. This heating step is not absolutely necessary if the alginate solution will be used within 24 - 48 hours. The heating step is recommended if the alginate solution will not be used within 48 hours. Refrigeration of the alginate solution should also prolong its shelf life.

The alginate solution should be cooled before dispensing into the 50 ml mixing chambers (see below). If the solution cannot be used within 48 hours of preparation it should be refrigerated. The alginate solution can be stored in the refrigerator in a sealed container for up to 15 days if it has been heat treated as described above. Allow the alginate solution to warm to room temperature before use.

Dispense 30 ml of the alginate solution into each of the 50 ml plastic mixing chambers. You can use the markings on the side of the plastic mixing chamber to measure volume. (Three of these mixing chambers can be used to make one 2 or 1.5 liter "lava lamp").

Advance Preparation of Calcium Chloride Solution:

Prepare a 5% $CaCl_2$ solution (5 g per 100 ml water; 1/2 tablespoon is approximately 5 g; 1 coffee measure or 2 tablespoons can be used to prepare 400 ml $CaCl_2$ solution). This solution only takes a few minutes to dissolve, stirring the solution will speed up the process. *Caution:* Avoid getting any calcium chloride solution on your hands otherwise it may dry your skin. Spilled calcium chloride powder or solution should be immediately rinsed from skin with plenty of water to prevent the skin from drying. Dispense (or have the students dispense) the 5% $CaCl_2$ solution into clear plastic tumblers or plastic glasses until about 2/3 full.

Advance Preparation of Sucrose Solution:

Prepare the 10% sucrose solution by mixing 100 g (approx. 1/2 cup) with 1000 ml of distilled or tap water. This can be done directly in the plastic bottle used for the lava lamp. Place the cap on the bottle and shake vigorously for a few seconds. Repeat a few minutes later. The sugar only takes a few minutes to dissolve. Make enough solution to fill soda bottles 3/4 of the way.

B. Students (each group):

Listen to and observe the precautions given by your teacher.

Spread some newspaper over your work area.

Allow alginate solution to cool to 41-46 ^{o}C (105 - 115 ^{o}F) if it is hot. Otherwise warm up the alginate solution to 41-46 ^{o}C (105 - 115 ^{o}F) (check with thermometer). Solution must not be warmer than 46 ^{o}C (115 ^{o}F) but must be warm enough (41 C) to activate the dried yeast.

Add 8.5 to 9.0 grams (approximately 1/2 level tablespoon) of glass powder to the 30 mls of alginate solution. Note to teachers. This measurement is fairly critical. If too much glass powder is used the beads may stay on the bottom, and if too little is used the beads may stay at the top. Unfortunately there is wide variation in commercially available measuring spoons. We suggest using a balance, if possible, or a least checking the type of measuring spoon you intend to use with glass powder and a balance and make adjustments accordingly. Alternatively teachers could provide 50 ml mixing chambers with pre-weighed glass powder.

Add 1 ml of Glo-Sperse pigment to the alginate/glass suspension using the plastic transfer pipette. (This step is optional. Your teacher may have you omit this step or may have you use a different type of color pigment). Cap the mixing chamber tightly and shake to disperse the glass and pigment in the alginate solution.

Note to teacher: If time does not permit yeast addition and bead formation in one class period, it is possible to delay yeast addition and bead formation until the next class period.

Add 1 gram (1/4 level teaspoon is approximately 1 g) of dried yeast to the 30 ml of alginate/glass powder/pigment suspension in the 50 ml plastic mixing chamber. The mixing chamber should be capped tightly and shaken to suspend and activate the yeast. Shake the closed chamber vigorously every few minutes. Let it sit for a while and then shake again. Repeat shaking to uniformly mix the suspension.

Pour the 5% calcium chloride solution into a clear plastic tumbler until it is about 2/3 full (your teacher may have already done this). Calcium chloride can dry out the skin. If any solution accidentally comes in contact with skin, please rinse the area thoroughly with plenty of water. Open the mixing chamber containing the alginate, glass and yeast suspension and fill the plastic transfer pipette with the suspension. Fill the pipette with the material in the bottom of the mixing chamber. This will help ensure the glass powder is more evenly distributed. Hold the pipette just an inch away from the $CaCl_2$ solution in the tumbler. Drip the alginate/yeast suspension into the $CaCl_2$ drop-by- drop. (Not too fast, but not too slow either; otherwise the glass will not stay evenly dispersed in the suspension). ***Do not touch the transfer pipette to the $CaCl_2$ solution at any time.*** Always hold the transfer pipette above the $CaCl_2$ solution.

Cap the mixing chamber tightly and shake to disperse the glass particles in the alginate/yeast/glass mixture again. The capped mixing chamber should be shaken each time before loading the pipette.

Repeat the dripping procedure and keep forming the beads until all of the alginate/yeast/glass/color suspension is used up.

Allow the beads to sit in the $CaCl_2$ solution for at least 5 minutes. Swirl the beads periodically during the 10 minutes. Avoid splashing the $CaCl_2$ solution on your skin. If you do, wash the solution off your skin with tap water.

Rinse the beads at least 4 times by decanting most of the liquid but not pouring out the beads. This helps remove excess $CaCl_2$ solution. Beads can be filtered with a tea strainer or piece of nylon stocking and washed with several volumes of water. Your teacher may have you save the $CaCl_2$ solution for reuse.

C. Students or Teacher:

Combine the rinsed beads produced from three of the 50 ml mixing chambers and add them to a 2 liter soda or a 1.5 liter water bottle. Smaller bottles such as a 1 liter, 500 ml or 250 ml water bottle can also be used, if desired. The use of a funnel which fits into the bottle opening will facilitate transfer. If multiple bottles are used , roughly equal amounts of beads should be placed in each bottle.

Insert the bubbler all the way into the rubber stopper. It may be easier to dip the narrow end of the bubbler in water before inserting it into the rubber stopper. Then add exactly 1 ml water to the top opening of each bubbler (fermentation airlock). The 1 ml volume can be measured with the plastic transfer pipette. Identical volumes in each bubbler is especially important if rates of bubble formation in one “lava lamp” will be compared to others.

Insert the rubber stopper with the bubbler into the top of the large bottle. Push down and twist the stopper to seal.

Securely attach a glove to a straw with a rubber band. Do the same with the mylar balloon. The straws are Glad brand 7 5/8” long X 15/64” diameter (the diameter is important for a tight fit into the bubbler). Approximately 2” of the straw should stick out from the bottom of the glove or balloon. After the yeast cells are actively producing gas bubbles, insert the straw (with the attached glove or balloon) into the top of the bubbler until it fits tightly.

Note to teacher: The mylar balloon will trap the CO_2 gas and this can be used to demonstrate some of the properties of CO_2 (e.g. CO_2 will extinguish a glowing splinter or the flame of a small candle set on the bottom of a glass beaker; CO_2 is also heavier than air). A more effective means of trapping CO_2 produced by the "lava lamp" is to attach some tubing to the bubbler and collect the gas using a pneumatic trough and gas collection bottles.

What happens:

When the yeast beads are added to the sucrose solution, they settle to the bottom of the bottle. As yeast activity develops (~ 1-2 hr), carbon dioxide is produced, gas bubbles form, and the buoyancy of the gas bubbles causes the beads to rise. to the surface. Eventually the CO_2 bubbles are released at the surface. The glass powder in the beads acts as a weight allowing the beads to sink to the bottom again.. The CO_2 travels through the fermentation lock and is collected in the glove or mylar balloon. As yeast metabolism continues, the rising and falling action of the beads gradually increases. At room temperature the activity will continue for several days. The rate of CO_2 production can be monitored by counting the bubbles which pass through the fermentation lock. It is often sufficient to count the number of bubbles formed in the fermentation lock for a period of 5 to 10 minutes and then calculating the number of bubbles per minute. Counting the number of bubbles for just 1 or 2 minutes may give inconsistent results.

Enhancing consistency of results:

To help enhance consistency in results among different lava lamps you might consider the following:

Have everyone use the same type of measuring spoons. The material being measured in each spoon should be leveled with a flat edge (for example a ruler). Alternatively a balance can be used if one is available.

The amount of water added to each bubbler (fermentation lock) should be the same (for example exactly 1 ml), otherwise the bubbling rate can vary from bottle to bottle.

Make sure that the fermentation lock is tightly inserted in the rubber stopper and that the stopper is tightly inserted into the bottle. It is a good idea to twist the fermentation lock in the stopper and to twist the stopper after it is inserted into the bottle.

Have students count the number of bubbles in the fermentation lock for a sufficiently long period of time (5-10 minutes) and then calculate the number of bubbles per minute. Counting the number of bubbles for just 1 or 2 minutes may give inconsistent or misleading results. Counting the number of beads rising past a certain point is another way to crudely measure CO_2 production rates, but given the variability of the beads, this method may not be very accurate or consistent. It is possible to trap the evolved gas using a simple pneumatic trough and gas collection bottles. It may take longer to determine CO_2 production rates, but this method allows students to measure the total amount of CO_2 produced and to test the gas produced with the glowing-splinter method.

The alginate/yeast beads prepared by all groups can be combined and mixed and then evenly distributed among the bottles. Randomized mixing should help reduce variability. For some experiments pooling the beads may not be desirable.

The alginate, sucrose and $CaCl_2$ solutions can be prepared in separate large batches and then distributed evenly among the groups. The volume of sucrose solution should be the same in all bottles.

Have each group check the temperature of their alginate/yeast suspensions with the same thermometer or with different thermometers that read the same.

Have each group use a graduated cylinder to measure volumes consistently.

Logistics:

If there is insufficient time to have the students prepare the alginate/yeast suspension and form the beads the same day, it is possible to mix the alginate, glass powder and pigment on one day, and then add the yeast and form the beads during the next class period. In this case, warming the alginate/glass/pigment suspension to 41-46 ^{o}C (105 - 115 ^{o}F), but not warmer, will help activate the dried yeast. Advance preparation of solutions and setting up student work areas with all materials needed will obviously facilitate the assembly of the lamps.

The microbial "lava lamps" can be made with a variety of plastic bottles including 2 L soda bottles, and water bottles ranging from 1.5 liters down to 250 ml. The important thing is that the rubber stopper and bubbler seat firmly in the mouth of the bottle. Experiments involving comparisons among lamps should be run with bottles of the same size and filled to the same volume with sucrose solution.

The microbial "lava lamps" should remain active for a week or two. After one or two weeks the sucrose solution can be replaced with a fresh solution. Collect the beads with a strainer and rinse with water. Replace the old sucrose solution with freshly-prepared 10% sucrose and return the beads to the bottle using a funnel. Replace the bubbler. Activity should resume within 24 hours.

Precautions:

The alginate, calcium chloride, sucrose and powdered glass are all white powders. Please read label carefully.
The alginate is provided in a small plastic container labeled "Sodium Alginate". The calcium chloride is provided in a 4 oz plastic container labeled "Calcium chloride". The glass powder is provided in a 4 oz plastic container labeled "Glass Powder".

The water bath used to heat-treat the alginate solution will be very hot (boiling). Teachers should have hot mitts or gloves ready for use when needed. Warm water will also be used to heat the alginate and yeast suspension so care must be exercised by teachers and students to avoid burns or spillage of hot water.

The calcium chloride powder or solution can cause drying of the skin so any spills should be rinsed off the skin with plenty of water.

Caution: The beads **are not edible** even though they contain yeast and the food ingredient alginate. The glass and the optional color pigment are not edible.

Do not breath the glass powder used in the experiment. Spilled glass powder should be vacuumed and the area wiped clean with several moistened paper towels. Do not breath dust from any of the powdered chemicals.

Carbon dioxide gas will be produced shortly after the immobilized yeast cells are placed in the sucrose solution. **Do not place beads containing yeast cells into a bottle that will be tightly sealed; the original bottle cap must not be used.** Use of the bubbler (fermentation airlock) either with or without an attached glove or balloon is appropriate, however the glove or balloon should be removed after it becomes fully inflated.

Ethanol (ethyl alcohol) will be produced naturally as the yeast cells ferment the sugar. The contents of the bottle must not be consumed at any time. As time goes on, an acetic acid (vinegar) smell may be noticed. This is from oxidation of the ethanol to acetic acid by naturally-occurring bacteria present in the apparatus. Care must be used when discarding the solutions to avoid contact with skin or eyes.

Glo-Sperse Color Pigments: The Glo-Sperse EPX pigments from Day-Glo Color Corp. have been pre-approved through Duke University for the Art and Creative Materials Institute (ACMI) certification program. These pigments are non-toxic pigmented acrylic microbeads suspended in water. The pigments are used in such items as artist Tempera Paints,

fluorescent highlighting markers, brightly colored papers and inks. However they will stain clothing if allowed to dry, so students should wear aprons or other protective covering. Day-Glo EPX Dispersions are essentially non-toxic and contain no constituent heavy metals or inorganic phosphorus. Contact with skin or eyes can cause irritation, so avoid contact with eyes and skin. First Aid: *Eyes:* immediately flush for at least 15 min. while holding eyelids open. Call a physician at once. *Skin:* immediately flush with water for at least 15 min.

The Glo-Sperse pigments contain a compound known to the State of California to cause cancer: Acrylonitrile (no more than 50 ppm, usually 5-10 ppm). Much of this chemical is immobilized within the pigment microbeads themselves and will be further immobilized within the alginate beads. Thus the chance of exposure to acrylonitrile is very, very low. Students should use gloves when handling the pigment or pigmented beads. They should wash immediately with soap and water if pigment gets on skin. The pigment should wash right off unless it has dried. Rubbing the skin while washing should help remove any dried pigment. The amounts used in this experiment, the use of gloves and the transfer pipette, and the short time period in which the pigments are used should not pose a problem. The use of the Glo-Sperse pigments is entirely optional and will not affect the scientific basis of the exercise.

Teachers who are uncomfortable having students handle the Glo-Sperse pigments should either use them in a teacher-prepared demonstration, substitute low-cost liquid Tempera paints (available at artist supply stores), or omit the use of colored dyes or pigments entirely. Tempera paints work fairly well although they are slightly harder to handle and slightly harder to disperse in suspension. They also contain binders and some other agents which *may* affect yeast activity. There are some "glow-in-the-dark" Tempera paints which will allow the production of "glow-in-the-dark" alginate beads within the "lava lamp" which can be faintly seen in a darkened room.

The best way to dispose of the pigments is to let them dry in the container (lid off) or absorb the liquid pigment onto newspapers or paper towels. Once completely dry, the pigment can be safely disposed of in the trash. Small amounts of pigment can be rinsed from the pipettes and containers with water. Make sure that the pigment is highly diluted before pouring in the sink and use lots of running water. This should only be done for small amounts of residual pigment. This process of disposal is similar to that used when cleaning up acrylic latex paint from paint brushes.

When finished with the lava lamp, the beads should be removed from the sucrose solution using a screen, tea strainer or nylon stocking. They should not be allowed to go down the drain. The sucrose and $CaCl_2$ solutions can be poured down the drain. The beads can be safely disposed of in the trash. The bakers yeast used in this exercise is not a health hazard and may be disposed of in the trash without additional treatment.

Student questions and inquiry-based extensions to the activity:

There are a number of variations on this activity which could form the basis of other hands-on inquiry-based studies especially based on the rate of carbon dioxide evolution:

Controls: One batch of beads could be prepared without yeast cells (yeast-cell free control). One apparatus could be set up with only water and no sucrose (substrate free control)

Rates of fermentation could be measured by counting the bubbles released in the fermentation lock for given periods of time at different temperatures, e.g. at 0 ^{o}C; 25 ^{o}C; 37 ^{o}C; 50 ^{o}C.

"Identical" units set up by different student groups could be monitored for rates of carbon dioxide production. Concepts of natural variability of the beads (affecting their size and density), variation in measurements, and elementary statistical concepts can be discussed.

The beads could be placed in solutions with different pH's, different salt concentrations or different types of sugars other than sucrose. Some other types of sugars may be expensive. In such a case, smaller lava lamps could be constructed from smaller bottles.

The beads could be pre-treated with different temperatures (e.g. boiling water, 80 ^{o}C, 60 ^{o}C for a given period of time (e.g. 5 minutes) before being placed in the sucrose solution - demonstrating cell and enzyme inactivation by high temperatures.

Various chemicals could be tested for their ability to reduce or enhance the rate of carbon dioxide production.

Ties to other branches of science and mathematics:

Alginate is a polysaccharide derived from a type of algae found in the ocean called kelp. Large areas of kelp growth form a "forest" providing a suitable environment and protection for many marine life forms. Alginate is also produced by some bacteria.

The formation of an alginate gel from a liquid solution is based on chemistry, polymer chemistry and electrical charge interactions (opposite charges attract, like charges repel) . The long carbohydrate polymer is soluble in water, but has many carboxylic acid groups (COOH) (see the figures). At neutral pH the COOH group loses a proton (H^{+}) to become the negatively-charged COO^{-} group. There are so many negatively-charged groups that they tend to avoid each other, helping the alginate polysaccharide stay in solution. However when positively charged calcium ions (Ca^{++}) are added, they interact with the negatively charged COO^{-} groups on the alginate causing the long strands of alginate to come together to form a large rigid network of rigid alginate polysaccharide molecules - a gel (see the figures). That is why the solid beads form when the liquid alginate/glass powder/yeast slurry is dropped into the $CaCl_2$ solution.

Alginate is often used as a thickening agent in foods and so is related to food chemistry and food science. Food scientists may use alginate, calcium ions, and other polysaccharide molecules to adjust the thickness or flow properties of a particular food product formulation. Alginate is also used in the pharmaceutical industry and for other industrial applications.

Students may ask why the beads are spherical. This would relate to some important concepts in physics and math and might be a question that a student or groups of students could research in the library, on the World Wide Web, or discuss with their physics or math teacher. [The answer is related to surface tension and the surface area/volume ratio of a sphere as well as the viscosity of the alginate solution. The surface area/volume ratio is also an important idea in microbiology - smaller single-celled organisms have a much higher surface area/volume ratio than larger cells and this allows them to be metabolically very active.]

Alginate-immobilized cells are often used in medical research, pharmaceutical applications, and microbial production of specialty compounds.

The carbon dioxide produced by the yeast can be trapped in a mylar balloon. The collected gas can be tested with the "glowing splinter" technique. A glowing wooden splinter will be extinguished by carbon dioxide but will ignite in the presence of oxygen. The carbon dioxide could also be collected, and its volume measured using an inexpensive pneumatic trough and gas collection bottle.

Some other questions students might consider or might come up with on their own include:

Why was a warm water solution used to dissolve the dried yeast?
Why was a very hot water solution not used to dissolve the yeast?
What is the purpose of adding the glass particles to the bead mixture?
Does adding the colored pigment affect the rate of yeast fermentation?
Why doesn't the yeast, pigment, or glass come out of the bead?
The calcium ions are pretty small - why don't they diffuse out of the bead, away from the alginate chains? Why doesn't the alginate bead fall apart in the sucrose solution which is low in calcium?
How does the sucrose get to the yeast cells immobilized within a bead?
How does the CO_2 get out of the bead? Why do CO_2 bubbles stick to the outside of the beads?
How much CO_2 must be produced by one bead in order to have it rise from the bottom?

What happens to the rate of CO_2 production if the "lava lamp" is placed at cooler (ice water) or warmer temperatures?
How come all of the beads don't rise to the top all at once?
How come some beads rise (or fall) faster than others?
What other things could be done or what other products could be made with alginate?

What other questions or experiments do your students come up with? We would like to know. Please contact Dr. Paul Tomasek; e-mail: paul.tomasek@csun.edu; phone: 818-677-3386; fax: 818-677-2034.

The alginate, glass powder, and fluorescent pigments are available from California State University, Northridge. For more information, contact Dr. Paul Tomasek; e-mail: paul.tomasek@csun.edu; phone: 818-677-3386; fax: 818-677-2034. In the future, these kit ingredients may be available from alternative sources as well.

The current addresses for manufacturers of specialized ingredients:

Keltone LV alginate	**Spheriglass glass powder**	**Glo-Sperse pigments**
Nutrasweet Kelco Co.	Potters Industries Inc.	Day-Glo Color Corp.
8355 Aero Drive	P.O. Box 840	4515 St. Clair Avenue
San Diego, CA 92123	Valley Forge, PA 19482-0840	Cleveland, OH 44103
800-535-2656	610-651-4700	216-391-7070

These companies sell their products only in bulk quantities and purchasing supplies directly from the manufacturer is probably not feasible for most schools. In some cases the manufacturers may be able to provide limited-quantity samples of these products. **Sigma Chemical Co. (St. Louis, P.O. Box 14508, St. Louis, MO, 63178; phone 800-325-3010) sells alginate in quantities as small as 100 g for a reasonable price.** The Sigma catalog number is A 2158 for Alginic Acid, Sodium Salt (low viscosity). The glass powder is available from companies that carry sand-blasting products.