**Ampli Biochemistry Kit: Introductory Level**

**Today we will be experimenting with several things:**

* Designing Ampli circuits for flow chemistry and chemistry modeling
* Learning about yeast life cycle, fermentation, and products
* Evaluating the success of a bioreactor
* Exploring the energy content of ethanol with a calorimeter experiment

**Materials**

Check that you have all of the following:

* Ampli bioreactor with fermenting yeast culture
* Set of Ampli blocks
* Forceps
* Plastic pipette droppers
* Hot Hands warming pad
* Food coloring
* pH strips and urinalysis strips
* Your classroom will have color sensors, SensorTags, and multimeters to share

**1. Introduction to Ampli**

Take a look at your Ampli blocks. They each have paper for flowing fluids inside, and interlocking frames like puzzle pieces. Design a circuit with a handful of blocks, and flow food coloring through it. What directions does flow occur? What pathways can you create? What do you notice about how different colors interact when they meet? You have lids for your blocks with symbols on them. Can you create a pattern between the symbols and the role the block serves?

Discussion Questions:

* In what ways does this system differ from traditional laboratory chemistry?
* How might this system be uniquely useful?
* Are there specific environments that this setup would be beneficial in?

Teacher Notes: Students can use a combination of food coloring and salicylic acid to lower pH in the same circuit. Remind them they can turn corners, and that flow can start on two ends, and meet in the center! Have them describe and get used to the flow pattern and timing on paper. Encourage labeling with the lids.

**2. Ampli Bioreactors with Yeast**

You will receive from your teacher a bioreactor full of yeast. Your teacher will tell you all about these bioreactors, yeast, and fermentation. The bioreactor you receive will either still have saran wrap around it or the saran wrap will have just been taken off. From what you learned from your teacher about fermentation why do you think saran wrap was used (take note of the four small openings near the lid of the bioreactor)? What do you think would happen if no saran wrap was used? The bioreactor you have received will contain a mixture of growth medium, sugar, water, and yeast. The growth medium used here is agar - which is a gelatinous medium that provides nutrients and allows the yeast to grow happily.

Teacher Notes: Fermentation in general is a process that converts sugars to acids and gases and produces usable energy from nutrients. Every organism has a process like this, known as cellular respiration, which is very efficient and requires sugar and oxygen. Many organisms, including yeast, bacteria, and humans, also have an alternate energy production pathway -- the fermentation pathway kicks in for energy production and use of sugars when a key reactant required for normal cellular respiration is unavailable. Most commonly this missing reactant is oxygen; for instance, in humans if you exercise too much and your muscle cells do not get enough oxygen, the muscle cells go through lactic acid fermentation and produce lactic acid: the chemical that makes your muscles sore after you exercise a lot!

In yeast, fermentation is very useful in the food industry, because it takes sugars and converts them to ethanol and carbon dioxide gas. If you put yeast in bread dough, the oxygen supply is limited and it uses fermentation to break down sugars, resulting in lots of carbon dioxide that makes bread bubbly and fluffy rather than flat like unleavened bread. If you let yeast ferment various other fruits and grains, you can use the ethanol to create alcoholic drinks.

Fermentation happens when yeast are warm enough to grow with nutrients, but oxygen deprived, and with lots of sugar to break down. It produces carbon dioxide gas and ethanol (and some amounts of methanol as well, which is just one reason you shouldn’t drink the bioreactors -- methanol is purified out of alcoholic beverages but is poisonous to humans).

Your bioreactor was filled with water, the growth media, sugar, and yeast, and then sealed and allowed to sit in a warm area for many hours. From what you have learned about fermentation why are these steps important? Do you think you could have just done a few, but not all, of the steps (for example, skipped the sugar entirely) and obtained the same results? Why? In a few minutes you will be able to compare your bioreactor with your teacher’s bioreactor. The teacher’s bioreactor was filled with the same ingredients but the sugar was added just before class - so it has not had time to sit for many hours in a warm place. Do you have predictions as to how your bioreactor will be different from the teacher’s bioreactor?

Teacher Notes: We suggest the students test the glucose content of both a fermenting bioreactor and a non-fermenting bioreactor. The fermenting one, if it’s been working long enough, should have less or even no sugar left! The non-fermenting one should have had the original amount of sugar added to simulate the fermenting bioreactor before incubation. Consider also pH -- yeast, like the majority of living organisms (though not all! Some rare organisms are adapted for survival in crazy conditions like extremely high temperatures, extreme chemical environments, and extremely acidic environments) likes a pH of somewhere around 7 best. Yeast won’t make substances with unfriendly pH on its own, but contamination and other processes during fermentation \*can\* sometimes cause the pH to fluctuate. If you see a major difference in pH between bioreactors, there may be some interesting environmental effects happening.

**Smell**

Before this class you might not have known much about yeast - but many people recognise yeast as an ingredient in bread. It is perhaps the most common way to hear about yeast. Yeast is considered a “raising agent” and is used in many leavened breads (the breads that are spongy) and is absent from unleavened breads (the breads that are similar to crackers). When you bake bread yeast eats the starch from your ingredients and “burps” out CO2. This CO2 forms little air bubbles in your dough with in turn give leavened breads their texture.

Even if you did not know yeast was used in bread you most likely can recognize the smell of happily growing yeast like that in your bioreactor. Yeast smells like bread - bread smells like yeast - because many breads are made with yeast. The distinctive smell of freshly-baked bread when you walk into a bakery is almost entirely due to yeast.

Teacher Notes: Yeast is a tiny, microscopic fungus that can eat sugar and release alcohol and carbon dioxide. A clump of yeast is made up of oval-shaped cells which propagate through budding. Budding is asexual reproduction where a new cell grows from a bud on the old cell and the new cell eventually breaks away from the old cell. Yeast and the applications to bread have a fascinating history. It is speculated than humankind was using yeast for both leavening bread and fermenting to produce alcohol before written languages were developed. Hieroglyphics from ancient Egyptians indicate that leavened bread and alcohol may have been present in Egypt more than 5,000 years ago.

Carefully remove the lid of your bioreactor and see if you can smell your bioreactor’s yeast growing.

Now, construct a branch from your bioreactor. You can do so in the same manner as with the food coloring but now you should add a ramp block to connect the bioreactor to the blocks as well as adding a paper to the bioreactor so that the contents of the bioreactor can get to the ramp.

\*In the following sections you will be looking for color changes on indicator papers and this can be done either with a color sensor or with the naked eye. There are a few color sensors that will circulate around the room (and you will be shown how they work and can use one) but you certainly do not need a color sensor to observe changes\*

Once you have your branch constructed test the pH of your bioreactor’s contents by placing a pH strip in a block and having the liquid flow to the strip. The pH strip’s color should change as it gets wet. When your pH strip’s color has changed compare it to the key of colors that your teacher has to determine the pH of your bioreactor. Next, do the same thing with the teacher’s bioreactor. Are the two pH’s different? From what we have told you about fermentation and the differences between your bioreactor and the teacher’s why do you think there is or is not a difference in pH? You also have access to salicylic acid which - as is evident from the name - is an acid and has a pH less than 7. You can add salicylic acid to your branch and see how it changes your pH readings.

Salicylic acid will dry out your skin over long periods of time and you should not ingest any but if you handle it with care there should be minimal risk. Avoid getting salicylic acid on your skin and if you do get any on your skin wash it off thoroughly with soap and water.

Teacher Notes: The pH strips may already be insterted, or may not. If they are not, you can do one of two things -- Carefully remove the lid of the desired block (this should be plenty safe) and lay the pH strip face up on the block on top of the paper, allowing flow to occur. Or, more guaranteed to work well, simply remove the lid when you are ready to test, and press the pH strip face down on the block paper, letting it absorb the liquid from the paper and test. You can simply dip pH and urinalysis papers into the bioreactor, being careful not to disturb the yeast too much.

You will also have strips of test paper that look like the pH papers but can test for glucose - sugar. Repeat your process for testing pH to see if there is a difference in glucose levels in your bioreactor as compared to the teacher’s. Why do you think the levels are or are not different? Also, smell the teacher’s bioreactor and compare the smell to your bioreactor’s. Are they different? Why do you think that is the case?

Discussion Questions:

* Why does yeast smell? Do you think this is tied into how it allows bread to rise?
* How do you think yeast as a raising agent was discovered?
* Can you think of other uses for yeast?

**3. Calorimetry Experiment**

Teacher Notes: (this might all become a demonstration depending on how approval for students working with flames turns out) Calorimetry is defined as the measurement of the amount of heat released or absorbed in a chemical reaction, change of state, or formation of a solution. We will be observing the heat of combustion by burning alcohol. There are many types of calorimeters but the first was an ice-calorimeter made by Antoine Lavoisier and Pierre-Simon Laplace in 1782. An ice calorimeter works by measuring the melting of ice of a known mass. Ice and water are popular for use in calorimeters because water has a well-known, high, specific heat and water is common and easy to work with.

In this experiment we will be using heat capacity to determine heat of combustion and identity of the alcohol we will burn. Heat capacity is the number of heat units needed to raise, by one degree celsius, the temperature of a body. Energy, when in the form of heat, is measured in joules (J). For reference a watt (W) is one joule per second. In the case of water, 4.184 J is the heat capacity - the energy necessary to raise by one degree celsius one gram of water. For reference: 1 mL water = 1 gram water. From the known volume of water and the change in temperature of the water you can determine the heat energy transferred to the water during combustion of the alcohol.

Your teacher will give you additional background information on calorimetry but - in a nutshell - calorimetry is a way to observe heat released or absorbed as part of a chemical reaction. A chemical reaction is when the molecules or ionic bonds in a substance change. For example, burning paper would be a chemical change while folding paper would be a physical change. Burned and unburned paper are fundamentally different in ways that are harder to reverse that folded and unfolded paper (typically chemical changes are harder - or near impossible - to reverse than physical changes). In our case the chemical reaction we are concerned about is the burning of alcohol. The alcohol will be burned and we will be able to quantify the heat given off by this change. The heat of combustion is the heat energy released when a compound is burned and is often used to help determine the identity of a compound. Water, a specific alcohol, or a peanut will all have unique heats of combustion.

**Safety**

You will be working with matches, burning alcohol, and metals in contact with flame that will get extremely hot. You can easily get burned and if you think something might have been in contact with flames or heat do **NOT** touch it. There is no good way to tell if your metal can is hot by looking at it so do not take any risks and just assume that it is always hot until a teacher can check it for you.

Teacher Notes: Reiterate frequently the fact that hot metal looks just like cool metal and underestimating the temperature of a metal is a very common way to get badly burned. Make sure that the students have everything setup correctly (the metal can held up by a lab clamp the correct height above the tin foil bowl, the tin foil bowl is complete and large enough to contain all flames and set on top of the lid, and the thermometer is taped or resting in such a way that it is not touching the bottom of the can) before you set the alcohol on fire. You do NOT want students tweaking anything above the flame as it is burning. Also - given the time of year of this program long sleeves are probably not going to be prevalent but if anyone has long sleeves or scarves or anything that could dangle over the flame have them remove or tuck in the article that is a hazard.

**Testing for Alcohol**

Your bioreactor should have some ethanol and methanol from fermentation but in order for the calorimetry experiment to work there needs to be enough of these products. Your teacher will test your bioreactor’s contents by placing a for inside the bioreactor, getting a bit of the liquid on the fork, and holding the fork over the flames to see if the liquid burns off quickly. Ethanol and methanol burn readily where water - which was added to your bioreactor - certainly does not. If the liquid burns off easily then you should be set to run the following experiment but if it does not you will receive a little bit of supplementary methanol.

Teacher Notes: If you find your class is having consistently poor results with finding ethanol, there are several things that may be going wrong. It may be, especially on Tuesday, that the bioreactors simply need to incubate longer at the temperature they have been kept at. You could transfer them somewhere slightly warmer, or simply wrap them and leave them be for another night. It may be that the yeast are obtaining oxygen -- check that the plastic wrap is forming a relatively close seal (it doesn’t need to be perfect, but no major breezes). It may also be, especially later in the week, that the yeast may have run out of sugar to process. Try adding some more sugar to the bioreactor every other day. It may also be that the pH of the bioreactor has been changed and the yeast are not healthy -- check the pH, and try adding a mild base or acid to adjust close to pH 7. Also later in the week, the yeast may have too much ethanol -- this shouldn’t be a huge issue for class, but if the yeast seem to be diminishing in density or smell, try removing some of the ethanol supernatant and/or adding water.

In any case, if your class feels they do not have enough ethanol to work with, pour about 2mL pure ethanol (a relatively pure drinking or cleaning alcohol acceptable if no other option) into the bioreactor for use during class. Be aware that this is not particularly good for the yeast, so you may have to keep them warmer to recover or remove some ethanol supernatant after class.

**Important Information and Reminders**

1. The metal will get hot so do not touch anything that could have heated up. You can very, very easily get burned so **EXERCISE CAUTION**.
2. It is important that when you are are taking temperature measurements that the thermometer does not touch the sides of the can. You want to measure the change in the temperature of the water and if your thermometer is touching the side of the can your measurement will not be accurate. You can either hold the thermometer in place or you can tape it down such that the bottom of the thermometer is NOT touching the bottom of the can.

Supplies

* Tin foil
* Can lids
* Lab counter tops or something to insulate the table
* Tweezers
* Metal can
* Lab clamps (the most common version has three prongs and a tall stand)
* Thermometer
* Tape
* Cotton ball

Set-Up for the experiment - see presentation for additional photos and instructions

1. Take your jar lid and make a bowl out of tin foil to rest on top of this lid. This bowl should be large enough for your cotton wool ball to sit in and with walls tall enough to contain any flames that could result from burning your cotton wool ball. Place the tin foil bowl over the jar lid and place both of these over a pot holder (or something similar) to protect your work surface..
2. Take your lab clamp (the one with three prongs) and set it up such that the metal can is held above the lid with the tin foil bowl underneath. The can should just barely be touching the top of your tin foil bowl.
3. Your teacher will give you a specific volume of water to put into your can. Once the water is in your can place the thermometer inside the can and record your starting temperature.
4. Take a cotton wool ball and using tweezers hold the cotton wool ball in the bioreactor such that is absorbs some of the clear fluid above the lower layer of yeast. Try to rotate the cotton wool ball and get the whole thing saturated with the liquid.
5. Slide the tin foil bowl and jar lid out from under the can and place your cotton wool ball in the tin foil bowl.
6. Have your teacher light the cotton ball and slide the bowl under the can.
7. Wait until the cotton ball has completely burned to read the final temperature of the water.
8. Determine the temperature change (final temperature minus initial temperature) and report this number to your teacher. You will, as a full class, compare your results and talk about what they mean.
9. Wait to touch your can or disassemble anything until a teacher comes by to verify that the metal has cooled down.

**4. Design a Chemical/Ethanol Plant**

With our small bioreactors and blocks it is clear what each component of this system does. Your teacher will show you pictures of very large-scale versions of our bioreactor set up and you will not be able to see what every part of the plant does.

Teacher Notes: A chemical plant is where chemicals are manufactured or altered typically on a very large scale. An ethanol plant is where components of the corn plant are processed in order to result in ethanol that can be used for many things. Ethanol is added to most types of gasoline.. Ethanol is added to gasoline because ethanol helps to oxygenate gasoline which in turn allows gasoline to burn more completely during combustion.

This activity is geared less towards understanding the intricacies of the steps and machines that make up the production path in an ethanol plant and instead this activity is geared more towards having the students think both creatively and logically as they design their own ethanol plant. You can have the students design systems/machines to execute the various tasks necessary in an ethanol plant and have a large discussion as a whole class about the various approaches the students took to tackling the same challenge.

They typical ethanol plant has five major components:

1. Corn storage and milling
2. Saccharification
3. Fermentation
4. Distillation
5. By-product processing

Corn storage and milling is when the corn is ground down into smaller pieces and stored before it begins the next steps. This finely powdered corn is put, along with enzymes, into hot water and then cooled in what is called saccharification. The next step is fermentation which you know all about now. Distillation is where the mixture is purified and this purification continues into by-product processing when methods are used to remove many by-products.

That summary leaves out just about all the physical details. Where are these steps happening? How would you go from one step to the next? How would you design a machine or system to complete the tasks of each step? How would you design an ethanol plant? Is there a way to design the plant in a way where you can understand what each component does just by looking at the plant? Use paper to sketch out your ideas and be ready to share them with the class.

After you design your own plan your teacher will show you what current ethanol plants look like and where and how all these steps happen.

Teacher Notes: At the end of class, make sure that the student bioreactors are sealed with plastic wrap, and any that you might want to keep a SensorTag on have one and it is connected to an app on a nearby device. You may want to keep the non-fermenting bioreactor unwrapped, or you could wrap it and allow it to ferment and inoculate a new non-fermenting bioreactor for the next day.

To inoculate, fill growth media tube with water (preferably sterile dH20 or bottled water, but tap water would also work). Shake until mixed, pour into bioreactor, and add about 2mL (height in the tube) of dry yeast to the bioreactor. Keep in a decently warm place (not near AC’s or in fridges, room temperature is okay) for 8-12 hours. Then, to start fermentation, add about a tablespoon of sugar (table sugar or lab glucose) and wrap with plastic wrap to seal in a low-oxygen environment. Keep in a decently warm place again for 8-12 hours.

If you wish to clean the Ampli blocks yourself:

\*\*If your set has been used with harsh chemicals, is preferable to do this in a lab or classroom setting with proper chemical disposal containers and a designated sink. Wear gloves and a lab coat.

Remove papers from blocks with tweezers, and if possible soak in water to dilute the chemicals and lower the risk of interaction with environment after disposal. Dispose of water and papers in an appropriate chemical disposal (not a regular sink). If you are unable to access a chemical disposal immediately, don’t soak the papers, simply remove them and store them in sealed plastic bags, like papers together, until you are able to access a proper chemical disposal.

\*\*Blocks containing food coloring, ethanol, and salicylic acid are all safe to dispose of in regular trash and sinks and do not require any dilution. Yeast and media is also safe to dispose of in a regular trash or sink.\*\*

Place blocks themselves in a bottle or bowl, add dish soap or all-purpose cleaner, and (over a chemical-safe sink) run water into the container, allowing the blocks to slosh around (like a homemade dishwasher or laundry machine). If available, spraying the blocks down with ethanol or isopropanol spray will help to remove things that don’t remove easily with water. It’s okay to mix blocks together for this wash step! There’s very little chemical on them and a lot of water.

The bioreactors can be rinsed and cleaned with soap and water without any special concerns.