**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?

**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.

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?

**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.

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.

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 or Demo**

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.

**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 fork 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 ethanol.

**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.

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.