Name _____ **Period _____**

AP Biology **Date _____**

LAB _____. FERMENTATION OF SUCROSE

(aka MAKING ROOT BEER)

All organisms need energy to live. **Cellular respiration** is the process they use to convert the energy stored in sugars into the quick energy of ATP. If oxygen is available, the mitochondria can perform their "energy generator" job and make a lot of ATP energy. This version of respiration is called **aerobic respiration** and it produces enough ATP energy to support large active, multicellular animals like you and me.

If oxygen is not available, large organisms cannot produce enough energy to survive. That's why we die if we cannot breathe. But even though there is no oxygen, some one-celled organisms can still digest sugars and make enough ATP energy to live and grow. This version of respiration is called **anaerobic respiration**. Anaerobic means "without oxygen". Anaerobic respiration is used by bacteria and fungi and is also referred to as **fermentation**. There are two types of fermentation:

- **lactic acid fermentation** which is used by bacteria (and how we make yogurt) and also occurs in muscle cells when they are oxygen-deprived like during a sprint race: **glucose → ATP + lactic acid**
- **alcoholic fermentation**, which is used by yeast (a one-celled fungus) and how we make beer, wine, bread, and many other foods:
  glucose → ATP + alcohol + CO_2

In this lab, we are going to explore **alcoholic fermentation**. Really? Yes, really!

When yeast break down glucose to make ATP energy they also make two waste products: alcohol and CO_2. Unfortunately for the yeast the alcohol eventually builds up and kills them. But unlike the yeast, humans see the alcohol as a desirable product — an entertaining beverage. We like it so much, we purposely give the yeast sugars to ferment so they make alcohol. The CO_2 the yeast also produce collects in the fermenting liquid and makes it fizzy. That's why we use the terms "carbonation" or "carbonated beverage". This is the old-fashioned way that soda was made, like root beer, birch beer, and sarsaparilla. And that's what we are going to recreate in this lab: producing carbonated root beer through the fermentation of sugar

HISTORY OF ROOT BEER:

Root beer was made by our ancestors by soaking Sassafras (a type of tree) root in water, and adding sugar and yeast (for carbonation). In the early 1900s however, scientists discovered that safrole, a chemical found in Sassafras root, was a carcinogen (cancer-causing agent) and human consumption has been banned. Now, a mixture of other herbs and spices makes up "root beer extract" which is what is now used to make homemade root beer.

MATERIAL

- empty 2 liter plastic bottle
- 2 bottles (.5 liter) spring water
- brewer's (champagne) yeast
- sucrose (sugar)
- root beer extract
- small cup & large cup
- funnel
- 10mL & 5mL graduated cylinder

PROCEDURE:

Each of you will be given a bottle of spring water. This will be the water that you make your root beer out of and you will pour your root beer back into these bottles to ferment. Your **team of two people** will be mixing your root beer in one empty 2 liter bottle and then after it is mixed you will pour your soda back into the 0.5 liter spring water bottles. Each student will have their own 0.5 liter bottle of soda at the end of this process.

The procedures listed below are measurements for **one lab team of 2 people**.

1. Obtain your **warmed** bottles of spring water. The bottles should be heated to about body temperature (37°C).

2. Measure 0.25g of dry yeast in a small beaker or cup. Add 50mL of the warm water from your spring water bottles to the yeast so it dissolves. Let stand for at least 5 minutes.

3. While the yeast is dissolving, measure 4 milliliters of root beer extract.

4. Also, measure 110 grams of sucrose (table sugar).

5. Get your clean, empty large 2-liter soda bottle. This will be your **mixing bottle**. First, pour the sucrose into the 2-liter bottle using a funnel.

6. Next pour the root beer extract into the same 2-liter bottle. Use some of the warmed spring water to rinse out the root beer extract from the graduated cylinder and add it to the 2-liter bottle.

7. Now add the dissolved yeast mixture to the same 2-liter bottle.

8. Now add the rest of the 2 bottles of warmed spring water into the same 2-liter bottle to dissolve the sugar and mix everything up gently. You now have enough soda for two people.

9. Pour the root beer mixture into your empty spring water bottles. **Do not fill the bottle all the way.** Only fill to the point that the straight sides of the bottle start to curve in to the neck. Give your teacher any excess root beer mixture to make some ”Tester” bottles.

10. Close the cap on your bottle **tightly** and hold it upside down for a minute to check for leaks. Label the **cap** with your **initials** and your **class period** on it.

11. Observe and describe the appearance of the root beer on your lab.

12. Give the bottle to your teacher. We will age the root beer for 2–3 weeks at room temperature in a dark place. After that we will refrigerate for 1 week.

13. Refrigeration will stop the fermentation process and kill the yeast — and stop us from producing alcohol (Sorry!). Be sure to check bottles every day for tightness, if they get too pressurized, they will burst.

14. After chilling, get your bottle, open carefully, pour a sample into a cup, and observe and record the appearance of the root beer. Now have a taste.

SUMMARY QUESTIONS

1. Describe the appearance of the root beer before the fermentation process.

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2. Why were the yeast necessary in this experiment?

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3. Why was the sucrose necessary in this experiment?

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4. Why did we heat the water?

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5. What is the yeast trying to do with the sugar?

_____

_____

6. What is the CO_2 to the yeast?

_____

_____

7. Why do we have to leave the bottle for a few weeks before we drink it?

_____

_____

8. Why do we want the CO_2 to collect in the bottle? _____

_____

9. What is the alcohol to the yeast? _____

_____

10. What is the alcohol to us? _____

_____

11. Write the formula for **aerobic respiration**.

_____

12. Are the yeast using aerobic respiration?_____

13. What process are the yeast using to make energy? _____

14. Could we survive using this same process? If not, why not?

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15. Briefly describe what fermentation is and where each of the ingredients you used in this experiment fit into the flow of the fermentation process.

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16. Write a simplified chemical equation to show the products and reactants of the version of **anaerobic respiration** called **alcohol fermentation**.

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17. Complete the following AP essay question.

Yeast cells are placed in an apparatus with a solution of sugar (a major nutrient for yeast metabolism). The apparatus detects bubbles of gas released by the yeast cells. The rate of respiration varies with the surrounding temperatures as indicated by the data below.

Temperature (°C)	0	10	20	30	40	50	60	70
Number of bubbles of gas produced per minute	0	3	7	12	7	4	1	0

(a) **Graph** the results on the axes provided. **Determine** the optimum temperature for respiration in the yeast.

(b) Respiration is a series of enzyme-catalyzed reactions. Using your knowledge of enzymes and the data above, **analyze** and **explain** the results of this experiment.

(c) **Design** an experiment to test the effect of varying the pH of the sugar solution on the rate of respiration. Include a prediction of the expected results.