Detection of the production of CO_2 by *S. cerevisiae* during sugar fermentation and preparation of acid-base indicator

This experiment was the winning entry for the 2020 IYCN public outreach competition.

Detection of the production of CO_2 by *S. cerevisiae* during sugar fermentation and preparation of acid-base indicator

Targeted education level(s): Elementary/ Middle/High School/University-level students or graduates.

Experiment summary:

- Extract the pigment that gives the characteristic color to purple cabbage.
- CO_2 is produced by *S. cerevisiae* during sugar fermentation.
- Use cabbage pigment as an indicator to detect CO_2 produced by *S. cerevisiae* in the aqueous solution.

Chemistry concepts:

- pH
- pH indicators
- biochemistry: fermentation Equilibrium (carbon dioxide-carbonic acid)
- atmospheric chemistry: global warming

Length: 30-45 minutes

Required materials:

- Scotch tape
- One small container (aprox. 250 mL)
- One funnel
- Bleach
- Scissors
- Balloon
- 2 teaspoon of Yeast (available in a grocery store for making bread or pizza)
- 2 teaspoons of sugar
- 3 containers (eg. cup)
- Strainer
- One wide container (eg. bowl or pot)
- One straw
- One bottle (aprox. 500 mL)
- 50 mL of Ethanol
- 1-2 red cabbage leaves

Lab techniques:

- There are no specialized lab techniques used in this demonstration.

Detection of the production of CO_2 by *S. cerevisiae* during sugar fermentation and preparation of acid-base indicator

Chemistry concepts for each age range

Cabbage pH indicator

- Elementary students: Red cabbage juice contains a pigment that is an "indicator" liquid which turns red (or pink) when it comes into contact with an acid (pH less than 7) like lemon juice, and it turns blue or blue-green when it comes into contact with a base (pH greater than 7) like bleach or soap.
- Middle/High School/Science faculty students or graduates: A chemical called flavin in cabbage acts as an indicator to show pH changes through color changes. The change of the pH in the indicator solution becomes evident as the color of the juice changes in response to changes in hydrogen ion concentration.

Production of CO_2 using yeast

- Elementary: As the yeast eats the sugar, it releases a gas called carbon dioxide. A very similar process happens as bread rises. Carbon dioxide from yeast fills thousands of balloon- like bubbles in the dough. Once the bread has baked, this is what gives the loaf its airy texture. The gas fills the bottle and then fills the balloon as more gas is created. The gas passes into the straw and changes the acidity in the red cabbage juice.
- Middle/High School/Science faculty students or graduates: The bread yeast *Saccharomyces cerevisiae* uses the sucrose of the sugar to produce energy, releasing ethanol and carbon dioxide gas. This gas moves up the bottle and blows the balloon up and moves within the straw. When the CO_2 reacts with the water in the container it produces carbonic acid ($H_2O + CO_2 \longrightarrow H_2CO_3$) which produces a change in the hydrogen ion concentration of the solution. This is evidenced by the cabbage pH indicator.

Learning objectives:

Everyone: As CO_2 is produced naturally (respiration, fermentation) or by anthropogenic activities (combustion of wood and other organic materials and fossil fuels such as coal, peat, oil and natural gas), it is released into the atmosphere, but generally we can't see it since it is a colorless gas. Here we can observe this natural production of gas by living organisms (yeasts) when the balloon is inflated or by the bubbles in the cabbage juice solution.

With the increase in the concentration of CO_2 in the atmosphere in recent years, due to anthropogenic emissions and deforestation, the oceans have become more acidic. Similar to the experiment carried out here, the CO_2 produced dissolves in the water and produces its acidification, which we can see in the experiment with the color change of the cabbage pH indicator.

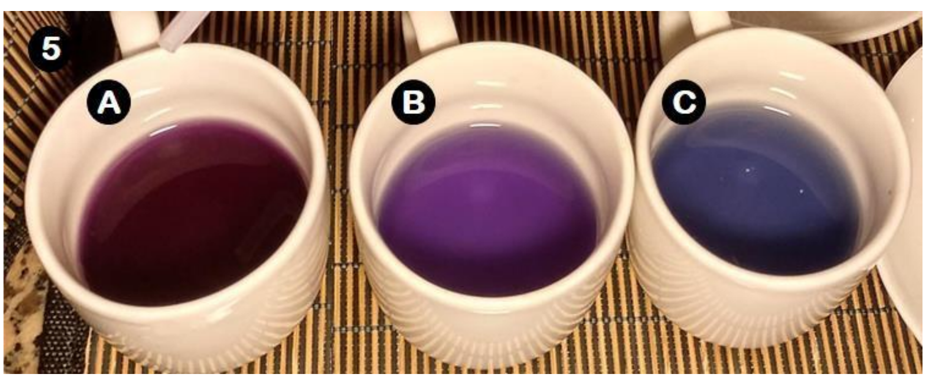
Experimental procedure:

1-2: Chop a cabbage leaf into small pieces and place them in a bowl.

3: Add 50 mL of alcohol measured in a food measuring cup

4: After crushing the cabbage, decant the prepared indicator (strong violet color) into a clean cup using a strainer.

5: Add drops of bleach and shake it turning it from violet to blue (From A to C).



A) Concentrated indicator obtained after crushing and strain
B) Diluted indicator with water
C) Indicator solution for use

1- After letting the yeast grow in the bottle submerged in warm water (approx. 10 minutes), CO_2 is produced. This gas inflates the balloon.

2- The gas goes through the straw to the container with the cabbage pH indicator (it is important to keep the end of the straw immersed in the solution all the time).

3- The gas is released in the solution, and some of the CO_2 reacts with the water producing carbonic acid, this makes the pH decrease (changing the cabbage solution color).

Note: It may be necessary to use boxes or another structure to support the experimental setup, as is shown in the images.

At the end of the experiment the indicator will change from blue to purple (more acidic than the beginning due to the dissolution of the CO_2 produced).

Experiment video available at:

https://youtu.be/XOq6w-wLa7U

Waste disposal:

- Once the bottle filled up completely, we moved the whole yeast sugar experiment to the sink, diluting them with water. Drain the pH indicator solution into the sink diluting it with water.
- The balloons and containers should be disposed of in the trash.

Teaching notes:

- Students and teachers can bring from home different substances to assess their pH with the pH indicator they made in the first step of the experiment.
- Teachers can propose changing the carbon (sugar) source used by the yeast in this experiment to see the difference in the rate of gas production. Examples of substrates: glucose, maltose, fructose. The gas production rate can be evaluated by measuring the time it takes for the indicator to change, using always the same proportions of the indicator solution. Also they can change the temperature of the water and evaluate the the effect of temperature on rate of gas production.
- You can also try not submerging the straw in the solution and see what happens with the indicator: how long it takes to change, that is to say, evaluate the dissolution rate (shift in chemical balance) depending on the amount of contact the gas and the aqueous solution have. (Note be careful not to have any gas missing in the second container, you can use another balloon).
- The production of CO_2 can be accomplished using baking powder (sodium bicarbonate: $NaHCO_3$) and vinegar or lemon juice instead of yeast, sugar and water. You can try this way by constructing the system and placing the bicaronate (2 teaspoons) in the balloon, and then letting the bicarbonate fall down into the vinegar (50 mL).
- The assembly of the experiment must be fast since the production begins quickly as soon as the container that contains the yeast is immersed in warm water, and we do not want to lose the gas produced.
- Most of the suggested materials to carry out the experiment can be replaced by laboratory materials (bohemian vessels, flasks, mortar, test tube, funnel). But because we are quarantined by COVID19 we had to transform our kitchen into a laboratory, this proves that the experiment can also be done at home.

Safety:

- Requires adult supervision—some household solutions can be poisonous when mixed together or swallowed.
- Students must not attempt to inflate the balloons with their mouths, especially after it is filled with the reacting agents.
- Do not do this experiment near the flame, because the pH indicator solution is flammable.

References:

- Madigan, M. T., Brock, T. D., Martinko, J. M., Madigan, M. T., and Martinko, J. M. Brock biology of microorganisms. Pearson, Boston, 2015. ISBN 978-0-321-89739-8
- Curia, M. V., D'Alessandro, O., & Briand, L. E. (2010). La Enseñanza de Conceptos en Biotecnología a través de un Experimento Sencillo y Económico. *Formación universitaria*, *3*(1), 27-30.

- Brown, T. L., LeMay, H. E., Bursten, B. E., Murphy, C. J., & Woodward, P. M. (2012). *Chemistry: The Central Science*. (12 ed.) Upper Saddle River, Nj: Pearson Prentice Hall.
- https://www.longdom.org/peer-reviewed-journals/research-fermentation-articles-17561.html
- https://www.exploratorium.edu/cooking/bread/activity-yeast.html
- https://sciencebob.com/blow-up-a-balloon-with-yeast/
- https://www.thoughtco.com/making-red-cabbage-ph-indicator-603650
- https://www.compoundchem.com/2017/05/18/red-cabbage/

- Student version -

Detection of the production of CO_2 by *S. cerevisiae* during sugar fermentation and preparation of acid-base indicator

Context: Carbon dioxide (CO_2) is a trace gas in Earth's atmosphere. Natural sources include volcanoes, hot springs and geysers, groundwater, rivers and lakes, ice caps, glaciers, and seawater. Also it is present in deposits of petroleum and natural gas. Plants, algae, and cyanobacteria use light energy to synthesize carbohydrates from CO_2 and water (H_2O), producing oxygen (O_2).

Aerobic organisms naturally produce CO_2 by respiration (energy production from carbohydrates and lipids metabolization). It is released to water via fish gills and to air via the lungs of air-breathing land animals, including humans. Also, CO_2 is produced during the processes of decay of organic materials and the fermentation of sugars in bread, beer, and wine making. Moreover, combustion of wood and other organic materials and fossil fuels such as coal, peat, petroleum, and natural gas produces CO_2 as a waste product.

The concentration of carbon dioxide has risen due to anthropogenic emissions, primarily from the use of fossil fuels and deforestation. Since the Industrial Revolution the atmospheric concentration of carbon dioxide increased by about 43%, leading (with other long-lived greenhouse gases such as methane, nitrous oxide and ozone) to global warming. Carbon dioxide also causes ocean acidification because it dissolves in water to form carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-})

All cells require energy to maintain their complex structures and to grow and reproduce. Cells obtain the energy they need by breaking complex foods (organic molecules) into simpler molecules. Some cells completely degrade food molecules like sugars into CO_2 and H_2O by a process called **aerobic respiration** (eq. 1). Some cells can only partially degrade sugars using a process called **fermentation**. Yeasts are unicellular fungi that can perform aerobic respiration when oxygen is plentiful, but they can also obtain energy by the anaerobic process of fermentation when it is not, for carrying out both kinds of metabolism they are chemoorganotrophs. When yeast cells carry out fermentation, they convert sugar molecules into ethanol (C_2H_5OH) and CO_2 (eq. 2). Yeast are of great economic importance. Their ability to carry out fermentation to produce ethanol is used in the production of alcoholic beverages such as beer and wine. In the production of bread, the CO_2 that yeast produce generates gas bubbles that cause dough to rise. During the proofing process, yeast mixes with the dough wet in the presence of a small amount of sugar, turning sugar in alcohol and CO_2, then the gaseous CO_2 expands and causes the dough to lift and fluff up. When the bread is cooked, heat expels the CO_2 and the alcohol, so they form holes within the dough producing its characteristic light texture.

Equations:

1. $C_6H_{12}O_6$ (aq) + 6 O_2 (g)→ 6 CO_2 (g) + 6 H_2O

2. $C_6H_{12}O_6$ (aq) → 2 CH_3CH_2OH + 2 CO_2 (g)

3. H_2O (l) + CO_2 (g) → H_2CO_3 (ac)

4. pH = -log [H^+]

5. H2CO3 (ac) ↔ $HCO3^-$ (ac) + H^+ (ac)

Humanity has been fermenting food since the Neolithic age, long before people understood the science behind the process. Today, following the scientific discoveries of French microbiologist Louis Pasteur, who showed that living organisms initiate fermentation, we know why fermentation not only makes food like sourdough bread, cheese, and wine taste better, but also helps to keep us alive.

Saccharomyces cerevisiae is the most common yeast used in bread and alcoholic beverages production, and it is generally sold dry and inactive in the supermarkets. When warm water and sugar (food source) are added, yeast become active and grow carrying out the fermentation process. As mentioned before, the CO_2 released in this process reacts with the surrounding water producing carbonic acid (eq. 3). Thus, gradually, the environment becomes more acidic. This change could be monitored using an acid-base indicator, also called pH indicator, a colored substance that varies its hue by changing the pH (degree of acidity) of the solution.

When an acid is dissolved in water, hydrogen ion (H^+) is formed increasing the solution acidity. Since the concentration of H^+ in an aqueous solution is usually very small, it can be conveniently expressed in terms of pH, since it offers more comfortable values for practical purposes. pH is defined as the negative base 10 logarithm of the concentration expressed in molarity (mol / L) of H^+ (eq.4).

Red cabbage contains a pigment molecule called flavin (an anthocyanin). This water-soluble pigment is also found in apple skins, plums, poppies, cornflowers, and grapes. Very acidic solutions will turn anthocyanin into a red color. Neutral solutions result in a purplish color. Basic solutions appear in greenish-yellow. Therefore, you can determine the pH of a solution based on the color that it turns the anthocyanin pigments in red cabbage juice. Thus, the color of the juice changes in response to changes in the H^+ concentration.

In brief, when CO_2 is produced (eg. fermentation process carried out by *S. cerevisiae*) and it is dissolved in water, it produces H_2CO_3, which releases H^+ (eq. 5) increasing the acidity of the solution (this is an equilibrium). This can be observed using the red cabbage pH indicator.

Learning objectives:

Cabbage pH indicator:

Learning Objectives by Age Range:

Elementary (Children or those with general science background): Red cabbage juice contains a pigment that is an "indicator" liquid which turns red (or pink) when it comes into contact with an acid (pH less than 7) like lemon juice, and it turns blue or blue-green when it comes into contact with a base (pH greater than 7) like bleach or soap.

Middle/High School/Science faculty students or graduates: A chemical called flavin in cabbage acts as an indicator to show pH changes through color changes. The change of the pH in the indicator solution makes evident as the color of the juice changes in response to changes in hydrogen ion concentration.

Producing CO_2 using yeast:

Learning Objectives by Age Range:

Elementary (Children or those with general science background): As the yeast eats the sugar, it releases a gas called carbon dioxide. A very similar process happens as bread rises. Carbon dioxide from yeast fills thousands of balloon- like bubbles in the dough. Once the bread has baked, this is what gives the loaf its airy texture. The gas fills the bottle and then fills the balloon as more gas is created. The gas passes into the straw and changes the acidity in the red cabbage juice.

Middle/High School/Science faculty students or graduates: The bread yeast *Saccharomyces cerevisiae* uses the sucrose of the sugar to produce energy, releasing ethanol and carbon dioxide gas. This gas moves up the bottle and blows the balloon up and moves within the straw. When the CO_2 reacts with the water in the container it produces carbonic acid ($H_2O + CO_2 \longrightarrow H_2CO_3$) which produces a change in the hydrogen ion concentration of the solution. This could be evidenced by the cabbage pH indicator.

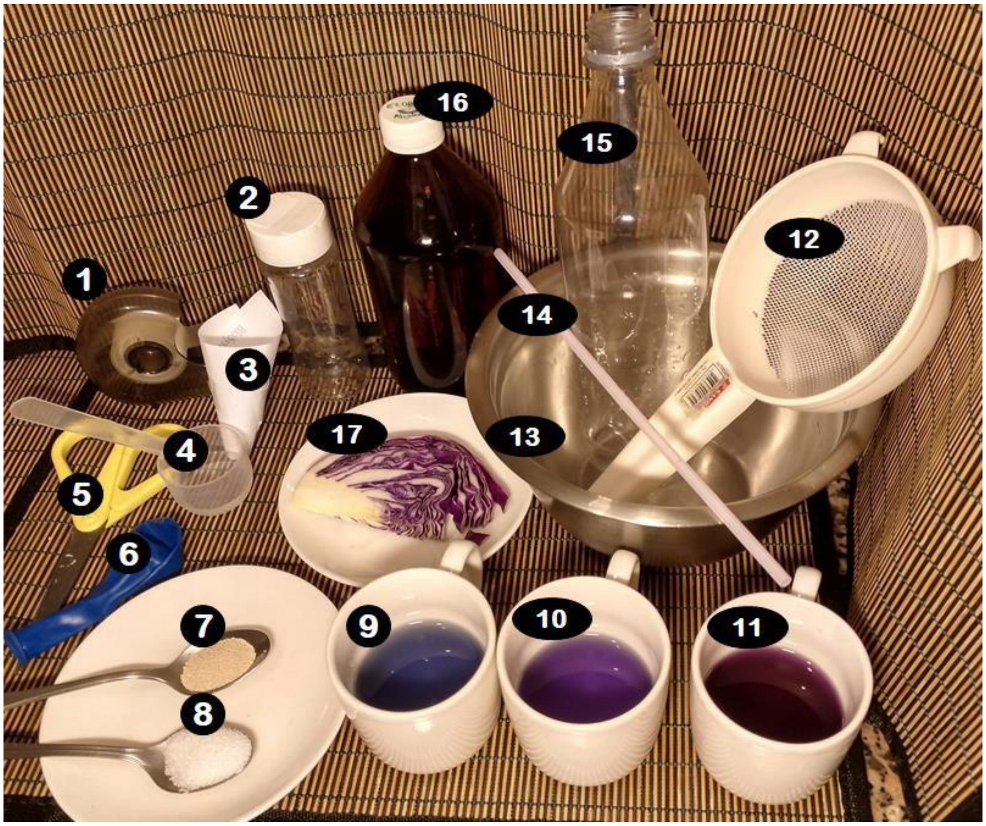
Entire experiment:

General Learning Objectives:

Everyone: As CO_2 is produced naturally (respiration, fermentation) or by anthropogenic activities (combustion of wood and other organic materials and fossil fuels such as coal, peat, oil and natural gas), it is released into the atmosphere, but generally we can't see it since it is a colorless gas. Here we can observe this natural production of gas by living organisms (yeasts) when the balloon is inflated or by the bubbles in the cabbage juice solution.

With the increase in the concentration of CO_2 in the atmosphere in recent years, due to anthropogenic emissions and deforestation, the oceans have become more acidic. Similar to the experiment carried out here, the CO_2 produced dissolves in the water and produces its acidification, which we can see in the experiment with the color change of the cabbage pH indicator.

Lab materials:



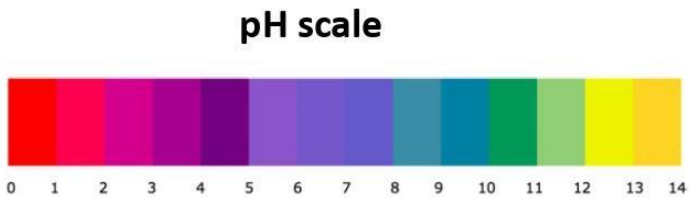
1- Scotch tape
2- Small container for the diluted blue indicator (approx. 250 mL)
3- Paper funnel (it will help to put the yeast and sugar in the bottle)
4- Bleach
5- Scissors
6- Balloon
7- Yeast (available in a grocery store for making bread or pizza)
8- Sugar
9-10-11- Containers for the different pH cabbage indicator solutions
12- Strainer
13- Container for keeping the warm water around the bottle
14- Straw
15- Bottle (approx. 500 mL) for growing the yeast
16- Ethanol
17- Red cabbage

Lab procedure or task:

- *Cabbage pH indicator:*

1) Chop a cabbage leaf into small pieces and place them in a bowl.
2) Add 50 mL of alcohol measured in a food measuring cup*.
3) Crush the cabbage until the liquid turns a strong violet color.
4) Decant the prepared indicator into a clean cup using a strainer.
5) Add drops of bleach and shake until the color turns blue.
6) Dilute the blue indicator with water in equal parts and drop it in a container.

*Note: In this step, the ethanol could be replaced by boiling the pieces of red cabbage in water for 30 seconds. Then let the solution sit for about 30 minutes to cool down. Then continue with step 4). We consider that the risk of this alternative is higher than the one proposed above.



- *Producing CO2 using yeast:*

  1) Make a little hole in the base of the balloon and introduce the straw and tape them together.
  2) Fill the bottle up with about 3 mL of warm water.
  3) Add the two teaspoons of the dry yeast and gently swirl the bottle a few seconds to mix the content.
  4) Add the sugar and swirl it around some more.
  5) Place the neck of the balloon over the neck of the bottle.
  6) Introduce the straw into the container with the aqueous solution with the pH indicator prepared before. It is important to keep the end of the straw immersed in the solution all the time.
  Note: It may be necessary to use boxes or something similar to support the experiment, as can be seen in the images.
  7) Let the bottle in a bowl with warm water for about 15-20 minutes (The temperature of the water in the container should be such that you can keep your hands under it).

Lab safety

Ethyl alcohol 70% - Highly flammable liquid and vapor, Can cause serious eye damage/irritation

Inhalation: If inhaled, move to fresh air. Seek medical attention if breathing problems develop.
Skin Contact: In case of skin contact, immediately remove contaminated clothing and wash affected areas with water and soap. Seek medical attention if symptoms occur.
Eye Contact: In case of eye contact, rinse with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Seek immediate medical attention.
Ingestion: If swallowed, do not induce vomiting. If vomiting occurs spontaneously, keep head below hips to prevent aspiration. Do not give anything by mouth to an unconscious person. Seek immediate medical attention.

Symptoms Caused by Exposure: Inhalation: May cause irritation to the nose, throat and respiratory system, dizziness, headache and possible confusion Skin Contact: May cause irritation to the skin, redness and itchiness. Eye Contact: Causes eye irritation, tearing, pain, stinging and blurred vision. Ingestion: May cause irritation to mouth, throat and gastrointestinal tract.

Discussion questions:

- Discussion questions:
  - Why does the gas change the pH of the solution?
  - What are other sources of sugar could *S. cerevisiae* use to obtain energy?
  - What do you think causes the color change of the indicator color?

Conclusion:

- When yeast ferments sugar and turns it into energy, they also produce carbon dioxide. This process is known as fermentation. In this activity, the balloons on the bottles should have captured carbon dioxide produced by the yeast during fermentation. As yeast carry out fermentation, the CO_2 they release reacts with the water to produce carbonic acid ($H_2O + CO_2 \longrightarrow H_2CO_3$). Gradually the environment becomes more acidic. The change in acidity can be monitored by using the cabbage indicator (change from blue to violet).