Biofuels
Enzymes for the production of bioethanol
KLA-110-265

Teacher's and student guide

Overview

In this series of experiments, students investigate the use of enzymes and microbes in the production of bioethanol fuel. Students first observe the ability of enzymes to break down starch. Students build their technical understanding and critical thinking skills by competing to design an apparatus to measure gas production during fermentation. They demonstrate their knowledge of biofuels, fermentation and enzymes by answering questions and evaluating their results. The kit is designed for 8 groups of up to 4 students.

Learning objectives

The students
- learn about alcoholic fermentation and the importance of enzymes in this process.
- investigate the process of ethanol production.
- consider how they can design and build an apparatus to measure gas production during fermentation.

Time requirements

- Day 1
  Preparation by teacher — 30 minutes
- Day 2
  Experiment preparation by teacher — 30 minutes
  Experiment by teacher — 40 minutes
  Groupwork by students
- Day 3
  Student group presentations — 30 minutes

Material

- Included in the kit:
  - Pancreatin powder
  - 75 ml 1% starch solution
  - 3x measuring spoons
  - 5x graduated pipettes
  - 30 ml iodine-iodine potassium (Lugol's solution)
  - 20x 15 ml centrifuge tubes
  - 1x pack of dry yeast
  - 16x packs of sugar
  - 16x 10-cm³ syringes
  - 16x end caps for syringes
  - 16x balloons
  - 32x plastic medicine cups
  - 32x wooden stirring sticks
  - 16x plastic bags
- Necessary but not included in the kit:
  - 1x water bath for a 250 ml beaker with 200 ml warm water (35-40°C)
  - 1x water bath for a 250 ml beaker with 200 ml hot water (80-90°C)
  - 8x thermometers
  - 1 liter wide-necked bottle with lid
  - 1000 ml graduated cylinder
  - 9x permanent markers
  - 9x alarm clocks
  - 400 ml warm tap water (for the yeast suspension)

    - Tap water (for the students during the experiment)
- Optional for conducting the experiment (not included in the kit):
    - Balance (accurate to 0.01 g)
    - 8x rolls of transparent adhesive tape
    - Measuring cylinder
    - Weighing dishes

Safety directives
Use this kit only in compliance with current safety regulations for laboratory activities, as well as suitable personal protective equipment.
Iodine-iodine-potassium solution leaves color stains on skin and clothing.

Background information
Yeast cells are often used in the production of bioethanol fuel. Yeast breaks down sugar and produces ethanol and carbonic acid as by-products. This process is called fermentation. Some plants store energy in the form of starch. Well-known examples include maize, potatoes, rice and wheat. Chemical or enzymatic processing of these sugar sources makes them available as raw material for yeast. Amylase, an enzyme found in pancreatic fluid, serves as a model in this series of experiments. Under suitable conditions, amylase splits starch into smaller sugar molecules.

Prior knowledge of the students
Before the students start the experiment, they should
- be familiar with the experimental set-up,
- know basic issues with regard to the environmental compatibility of fossil fuels,
- know the by-products of yeast fermentation.

Preparations by the teacher
- Day 1
    1. Copy the sheet "Biofuels contexts" for each pupil.
    2. Make a copy of the "Student experiment guide" for each group of students (or each student).
    3. Prepare two water baths, each with a 250 ml beaker.
    4. Assemble the material for the experimental set-up:
        - Pancreatin powder
        - 30 ml iodine-iodine potassium
        - 75 ml 1% starch solution
        - 4x thermometer
        - 3x measuring spoon
        - Permanent marker
        - 4x 15-ml centrifuge tubes
        - Alarm clock
        - 5x graduated pipettes
- Day 2: Experiment preparation
    1. Before class begins, prepare a water bath with 35-40°C warm water and a water bath with 80-90°C hot water. A 250 ml beaker with 200 ml of water is sufficient for each water bath.
    2. Hydrate the yeast for 15 to 30 minutes before the students start their experiments.
        - Fill 400 ml of warm tap water (35-40°C) into a 1-litre wide-mouth bottle. Attention: temperatures above 40°C destroy the yeast.
        - Add the yeast.
        - Close the bottle. After carefully shaking 15 times, the yeast should be well mixed and evenly distributed in the suspension.
    3. Shake the yeast again and then immediately pour 25 ml of yeast suspension into a plastic medicine cup for each group of students. Any remaining yeast can be kept

for further use during class. After class, excess yeast suspension should be poured down the drain.
4. Hand out the sheet "Biofuels contexts" to each student.

Performing the experiment

1. Have students observe or assist with each of the following steps.
2. Label four 15 ml centrifuge tubes A, B, C and D respectively.
3. Break down each solution on the board as follows:
   A. Starch 35°C
   B. Starch + pancreatin 35°C
   C. Starch 80°C
   D. Starch + pancreatin 80°C
4. Shake the 75 ml 1% starch solution vigorously.
5. Add 5 ml of starch solution to each test tube.
6. Place the uncapped tubes A and B in the 35-40°C water bath.
7. Place the uncapped tubes C and D in the 80-90°C water bath.
8. Heat the starch solution for about 5 minutes. Use this time to talk about the importance of enzymes in chemical reactions. Explain that pancreatin is isolated from the digestive tract of mammals, including humans. Pancreatin contains amylase, an enzyme that breaks down starch. You may also want to point out that human body temperature is about 37°C and that at high temperatures the enzymes can break down or become denatured.
9. Place a thermometer in each tube to ensure that all samples are in the correct temperature range. Add hot water to the beakers if needed.
10. Add one level measuring spoon of pancreatin powder to tubes B and D.
11. Close all four tubes and shake each one several times to mix the contents. Open the tubes again. Put each tube back into the corresponding water bath.
12. Allow another 5 minutes of reaction time.
13. While the reaction is going on, explain that iodine is a starch indicator. If the sample turns dark brown or purple, starch is present. If the solution is yellow or clear, starch is not present.
14. Using a pipette, add three drops of iodine-iodine potassium to each tube.
15. The students observe the changes at each rehearsal.
16. Discuss the reactivity of iodine with starch. The complex sugar molecules have been split into smaller sugars that do not react with iodine. At high temperatures, the pancreatin is denatured and has no enzymatic effect.

Experiment procedure – group work

1. Form eight groups of up to 4 students and hand out the "Student Guide to Experiments" to each group (or student).
2. Explain the objective in designing their apparatus. Show the students the material they will be working with and the material to be shared by all groups. Point out the list of materials in the student guide. All groups should have the same material at their disposal.
   - 25 ml yeast suspension in a beaker
   - 4x wooden stir sticks
   - 2x packs of sugar
   - 2x plastic bags
   - 2x 15-ml centrifuge tubes
   - Tap water
   - 2x 10 cm3 syringes
   - Permanent marker
   - 2x end caps for spray transparent tape
   - 2x balloons
   - Alarm clock
   - 2x plastic medicine cups

    - 1x Thermometer
3. Help the students to design their apparatus and plan the fermentation conditions, including the amount of yeast solution and sugar solution they will use.
4. If necessary, guide students in their design proposals by explaining the displacement of a liquid by a gas or the method of measuring volume by displacement. Examples of fermentation apparatus are shown below.
5. Instruct students to complete and hand in their draft plans.

Examples of fermentation apparatus

- Option 1
  Material: centrifuge tube, medicine cup, yeast, sugar, water, short time meter
  Procedure:
  1. Fill the centrifuge tube completely with a mixture of yeast suspension and sugar solution.
  2. Put the medicine cup over the tube.
  3. Turn the beaker and tube over quickly so that the beaker can stand on the table. The tube should be turned upside down inside the cup. A small bubble appears in the tube.
  4. If you use 10 ml of yeast suspension and 7 ml of the 10-20% sugar solution, gas formation occurs within 15 minutes. The students can read off the amount of carbon dioxide produced from the graduation marks on the tube.
- Option 2
  Material: plastic bag, permanent marker, transparent tape, yeast, sugar, water, timer, measuring cylinder (optional)
  Procedure:
  1. Tape the bag shut at one end to prevent leakage.
  2. Fill the yeast suspension and sugar solution into the bag.
  3. Remove excess air and tape the open end of the bag shut.
  4. If you use 10 ml of yeast suspension and 7 ml of the 10-20% sugar solution, gas formation occurs within 15 minutes.
  5. Measure the volume of gas by observing the change in volume in the bag. When students first add the liquid, make a line on the bag to indicate the initial volume in the bag. Once fermentation occurs, make a line every 5 minutes to indicate the total volume of gas in the bag. Find the difference between the initial and final volume and divide the difference by the elapsed time, then get the fermentation rate. Alternatively, students can find out the volume of the bag and its contents, by measuring the displacement of water in a measuring cylinder. Immerse the bag in a measuring cylinder and record the water level. The displacement can be measured at the beginning and at the end, or the bag can remain submerged during the entire fermentation time.
- Option 3
  Material: syringe, syringe end cap, yeast, sugar, water, timer
  Procedure
  1. Add yeast suspension and sugar solution to the syringe and remove excess air. The amount of liquid in the syringe should be less than 8 ml so that the students can measure the gas production.
  2. Place the end cap firmly on the syringe.
  3. Hold the syringe sideways or with the tip down so that the plunger moves when pressure is built up in the syringe.
  4. If you use 5 ml of yeast suspension and 3 ml of the 10-20% sugar solution, gas production occurs within 15 minutes.
  5. Measure the initial volume in the syringe and repeat the measurement at specific time intervals. Students can then use this data to calculate the fermentation rate.
- Option 4
  Material: centrifuge tube, balloon, yeast, sugar, water, short time alarm clock
  Procedure:

1. Fill the centrifuge tube completely with a mixture of yeast suspension and sugar solution.
2. Make sure that there is no air in the balloon. Put the balloon over the tube.
3. As soon as fermentation occurs, the balloon fills with gas.
4. Measure the volume of air in the apparatus by inverting the balloon and the tube and then reading the graduation marks at the beginning and at specific time intervals. Students can use this data to calculate the fermentation rate.
5. If you use 10 ml of yeast suspension and 7 ml of the 10-20% sugar solution, gas formation occurs within 15 minutes.

Day 2: Group work: Experiment procedure

1. First approve each group's design plan and check it for safety before allowing the group to start building and testing their apparatus.
2. Provide each group of students with a cup of yeast suspension.
3. Assist with setting up the apparatus and testing if required.
4. Give the students 30 minutes to test their apparatus. There is enough material so that the students can build two apparatuses and try them out at the same time. Give the students a start time and an end time.
5. Moderate the competition between the groups of students.
6. Have your students answer the rest of the questions.

Day 3: Group work

Presentations of the group results

Answers to questions in the student guide

- Questions about the context
  1. What is a biofuel? *A biofuel is an energy carrier produced from plant material, such as grain, or from another fibrous biomass.*
  2. Which aspects are decisive for research into the use of biofuels instead of or as a supplement to fossil fuels? *There is concern about the diminishing reserves of fossil fuels, which are non-renewable sources of raw materials. In addition, the production and use of fossil fuels causes a variety of environmental problems, both air, land and water pollution. Biofuels are produced from potentially renewable energy sources and have a lower environmental impact.*
  3. Describe two possible disadvantages of using ethanol as a biofuel on a large scale? *Problems include the loss of forest land as it is converted to cropland and used to grow commodities, increased soil erosion, increased use of pesticides and herbicides, and species extinction. Large-scale ethanol production can also lead to the decline of certain food crops (e.g. corn), as food crops could grow in the same field where feedstocks are grown.*
- Questions about the experiments
  1. (Experimental set-up) In which test tube did starch digestion occur? How do you know? *Starch digestion took place in tube B. Starch is not present, as indicated by the lack of blue/black colouring in the tube. Therefore, it must have been decomposed.*
  2. (Experimental approach) Which temperature range was more suitable for the digestion of starch? Why is this so? *The lower temperature range, 35-40°C is more suitable for starch digestion because it is most similar to the mammalian digestive tract where the enzyme naturally occurs. Amylase breaks down starch effectively at 40°, and the body temperature is about 37°C. At temperatures of 60°C and above, as in the hot water bath, amylase is denatured and no longer breaks down starch.*
  3. (Experimental procedure) Describe the conditions under which you carried out the yeast fermentation. *Different answers are possible.*
  4. (Experimental procedure) How would you change the apparatus or the conditions for the fermentation to further optimise the fermentation and produce more carbon dioxide? *Different answers are possible.*

5. (Experimental procedure) How do you think your apparatus and fermentation conditions differ from industrial mass production of ethanol? *Biofuels can be made from a variety of feedstocks. Corn starch is a popular feedstock that we used as a model in the demonstration. The experiment used yeast to break down glucose and produce ethanol in a reaction chamber, but the amount produced in our experiment was very small compared to the scale of production in the industry.*

6. (Experimental procedure) Suppose a 20 ml solution with 0.5 g yeast and 3 g sucrose produces 6% or 1.2 ml ethanol. How much yeast and sucrose would be needed to produce one litre (1,000 ml) of ethanol? *First determine the factor to convert 1.2 ml ethanol into 1,000 ml ethanol: 1,000/1.2 = 833.33. Then calculate the required amount of yeast: 0.5 g yeast x 833.33 = 416.67 g yeast. Finally, calculate the amount of sucrose needed: 3 g sucrose x 833.33 = 2,499.99 g sucrose.*