Biomass to sugars lab

Which feedstock will produce the most sugar?

Materials

- Glucose, ground up (3.0 g per station)
- Sweet corn, fresh or frozen, ground up (3.0 g per station)
- Cracked kernel corn, ground up (3.0 g per station)
- Test tube holder/hot pads
- 500 ml beakers
- Amylase enzyme solution (1 tsp amylase / 100 ml water)
- Glucoamylase enzyme solution (1 tsp glucoamylase / 100 ml water)
- Mortar and pestle
- Hot plates
- Stirring rods
- Water
- 15 ml centrifuge tubes (10 per group)
- Test tube racks
- Glucose test strips
- *Optional: Glucose monitor and test strips*
- Scale and weigh boats
- .5 ml disposable pipettes
- Marker/tape
- Scale
- *Optional: Stopwatch*
- *Optional: Ice bath*
- *Optional: Warm water immersion bath*
- *Optional: Pipette pump with 10 ml serolgical pipettes*

Directions

Procedure for all samples.

1. Each group needs:
   - 1 test tube rack
   - 10 test tubes
   - Marker
   - Stir rod
   - Glucose, ground up
   - 2 types of corn, ground up (sweet, dent)
   - Hot plate
   - 500 ml beaker that is half full of water
   - .5 ml disposable pipette.
   - Access to water, glucose test strips *or glucose monitor and test strips,* enzyme complexes and a scale are needed.
2. Use your marker to label your tubes according to Data Table 1.
3. Using the marks on the side of the test tube for guidance, pour 10 ml of water from your beaker into each test tube. Then return the test tube to the rack.
4. Bring the rack and test tubes to the weigh station. Place 1.0 g of glucose or ground corn into each of the appropriate labeled test tubes. Note that the enzyme only tube does not get any glucose or corn. When you are done, put the caps back on all 10 tubes.

Heat pre-treatment

5. Make sure your beaker is half full of water and place it on the hot plate until it begins to boil.
6. Loosen the caps on the test tubes labeled “heat.” Carefully place the 3 test tubes labeled “heat” in the beaker of boiling water. Allow samples to sit in the gently boiling water for 10 minutes. *Reminder: If it appears that water may begin to spill out, turn the heat down.*
7. Once the test tubes are cool enough to touch, carefully tighten the caps on the tubes and cool the heated tubes by running them under cool water or placing them in an ice water bath. Once samples have cooled to room temperature, move on to step 8.

Enzyme addition

8. Add .5 ml of Amylase an Glucoamylase solution to *glucose/heat, glucose/ no-heat, sweet corn/heat, sweet corn/no-heat, dent corn/heat, dent corn/no-heat* and *enzyme only* test tubes.
9. Cap and swirl to mix gently, then proceed to step 10.

Sugar measurements

10. To measure sugar, dip 1 glucose strip in a tube for 1–2 seconds. Be sure that the entire pad on the end of the strip is submerged in the solution. *Alternately, If a glucose monitor is present, place a test strip in the machine and turn it on, use a pipette to place a drop of solution on the end of the test strip at the prompt, read and record measurement at the prompt.*
11. Remove the test strip from the sample and gently drag it across the lip of the test tube to remove any excess liquid. Then prop the test strip up on its side so the liquid runs off the strip instead of pooling on the test area, and let sit for 1 minute. Next, one team member can dip a new test strip for the next test tube and start timing 1 minute. While the other team members move on, be careful to keep track of which strip goes with which tube.
12. Compare test strips with the color chart on the bottle and record your results in the data table. Be sure to do this right after the initial 1 minute is over because the color change on the glucose test strip will be inaccurate after 2–3 minutes.
13. Repeat steps 11 and 12 until your team has measured and recorded sugar levels for the samples in all 10 test tubes. *Alternately, if a glucose monitor is present, place a test strip in the monitor and turn the machine on, use a pipette to place a drop of each additional solution to the end of a new test strip and record the measurements.* Once you are done testing the glucose levels, place the caps back on the test tubes and loosely tighten.
14. Incubate at room temperature overnight, or, for optimal enzyme function, place in an incubator or water bath at 50–60° C overnight.

Data table 1

Tube no.	Label	Glucose amount day 1: time 0	Glucose amount day 2: time ___ hours	Glucose amount day 3: time ___ hours
1	Glucose / heat / date			
2	Glucose / no heat / date			
3	Glucose / no treatment / date			
4	Sweet corn / heat / date			
5	Sweet corn / no heat / date			
6	Sweet corn / no treatment / date			
7	Dent corn / heat / date			
8	Dent corn / no heat / date			
9	Dent corn / no treatment / date			
10	Enzyme only / date			

Day 2 and 3

15. Measure sugar levels the same way you did in Section D (Sugar Measurements) for all samples during your next 2 classes. Before taking sugar measurements, check that cap tubes are tight and gently swirl each tube for a few seconds to mix. Then measure and record the amount of sugar in all tubes using the glucose strip, or *glucose monitor*, as you did on Day 1.

Reflection

1. How much sugar is available in each feedstock for ethanol production?
2. Based on your findings, discuss why dent corn is currently being used to produce commercial ethanol? Support your conclusion with evidence gathered during the experiment.
3. What other pretreatment experiments could you do to assist enzymes in breaking down complex carbohydrates?