EXPERIMENT 5: Bioethanol Production from Yeast Fermentation of Sugars (activity 1G).

Learning outcomes: By the end of the session students should be able to:

- Describe the production of ethanol from renewable sources
- Describe the process of fermentation
- Carry out fermentation to produce ethanol
- Analyze the rate of fermentation of different sugars
- Evaluate the use and economic advantages of producing liquid biofuels (gasohol) from sugar

Keywords Bioenergy, biofuel, sustainable, renewable, biomass, yield, bioethanol, microbes, yeast, enzyme, fermentation, sugar.

Background Information:

Bioethanol is most often produced by fermentation of sugars by yeast. Ethanol tolerance is usually the limiting factor for ethanol production. The bacterium Zymomonas mobilis is a promising alternative to yeast due to its greater sugar intake, yields and resistance to ethanol concentrations.

The material used for bioethanol production includes sugar from sugar beet and sugar cane as well as starches from maize or grain feedstock. In this case the starches are first hydrolyzed with amylase enzymes (saccharification) to produce sugar that can be fermented by yeast. The yeast Saccharomyces cerevisiae produces ethanol by fermentation of sucrose or glucose but is unable to ferment pentose (C5) sugars. Saccharomyces diastaticus is able to utilize starch for fermentation. Research on the abilities of yeast to ferment different feedstock and sugars is essential to the development of industrial bioethanol production. The recovery and reuse of resources is important in making biofuel production economic and environmentally friendly.

In this lab you will compare the fermentation rates of yeast under a variety of conditions.
In order to calculate the rate of fermentation the amount of carbon dioxide produced can be measured over time. This can be done in a number of ways including:

- Collection of carbon dioxide (CO₂) in inverted lime water-filled measuring cylinders
- With a mylar balloon attached to the next of the conical flask or by tubing: The volume can be measured by carefully tying off the balloon used to collect the gas produced, immersing it in a large measuring cylinder and measuring the displaced volume, or by weighing the balloon, as the CO₂ is relatively dense.
• Using a Vernier CO₂ probe attached to the reaction vessel

You can also investigate a number of variables that affect the rate of fermentation. Some possibilities include strain of yeast; glucose concentration; temperature; pH; agitation.

Time: Approximately 2 session, 30-50 minutes

Materials:
• Conical flask (100 ml) or boiling tubes
• Boiling tube rack
• 8% (w/v) sugar solutions (Glucose, Sucrose, Fructose, Lactose, Maltose)
• 0.1 M phosphate buffer pH7
• Brewer’s or Baker’s yeast (*Saccharomyces cerevisiae*)
• Deionized or distilled water
• Stirrers
• Mylar balloons or bubble counters
• 50-ml graduate cylinder
• Thermometer

Method:
• Prepare the fermentation stock solutions in phosphate buffer
• Label six (6) conical flasks; add the yeast, sugar solution and buffers.
• Stopper the flasks with bungs holding fermentation locks and attach balloons to the neck of the flask or boiling tubes

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Analysis, Discussion, and Conclusions:

To organize and draw conclusions from your data, it is helpful to compare changes in glucose and ethanol levels over time using bar graphs. Using a computer program such as Microsoft Excel (or by hand), create two bar graphs to summarize your results. The empty graphs below can serve as a guide. Discuss the graphs with your lab group.

• Do these results match your initial prediction? Why or why not?
• How can you explain your results?
• Explain, summarize and communicate your results as instructed.