**Note to instructors:***Make sure to follow your local institutional guidelines with regard to safety in setting up this sort of chemical reaction, which may include the requirement that the materials be stored in a fume hood.  If you do not feel comfortable knowing you can conduct the experiment safely, you should not attempt it.*

**Experiment: Production of ethanol from sugar and methane production from manure**

Both sugar and manure (dead plant material or animal feces) contain much energy. But to make that energy most useful requires the action of microbes. In the case of sugar (sucrose), yeast obtain energy for their own needs by the process of fermentation, and as a consequence produce the useful waste product, ethanol. Ethanol can be used to run engines.

Manures left to decay in an anaerobic environment produce the very useful gas methane. Methane (CH4), the primary constituent of natural gas, can fuel engines, cook food, or heat houses. Less useful gases are also produced by the decaying manure, carbon dioxide, and hydrogen sulfide (H2S). Note that H2S is odorless, but quickly changes to SO4 in the presence of free oxygen. SO4 smells like rotten eggs.

These experiments will be done in discarded plastic soda bottles. The volume of reagents is based upon 1-liter bottles and can be modified for larger or smaller bottles. You start the experiments and let them run for one week.

**Ethanol Experiments**

1. The effect of yeast on the rate of production of ethanol.

Obtain four plastic 1-liter soda bottles. Label two bottles yeast, and the two others no-yeast.

To each add 125 ml (1/2 cup) of table sugar (sucrose), and 15 ml (tablespoon) of whole-grain flour (wheat or other). In the **yeast** bottles also add 2.5 ml (1/2 teaspoon) of yeast. Do not add yeast to the no-yeast bottles. Add 750 ml of tap water to each bottle, cap, and shake vigorously. Place the bottles at room temperature (22 deg C).

1. The effect of grain on the rate of production of ethanol.

As in experiment #1, obtain four soda bottles. Label two grain and two no-grain. All four bottles get the 125 ml of sugar 2.5 ml of yeast, but only the grain bottles get the 15 ml of grain. Add 750 ml of tap water to each bottle, cap, and shake vigorously. Place the bottles at room temperature (22 deg C).

1. The effect of temperature on the rate of ethanol production.

Obtain four plastic 1-liter soda bottles. Label two bottles 22 deg C , and the two others 4 deg C. To each add 125 ml (1/2 cup) of table sugar (sucrose), and 15 ml (tablespoon) of whole-grain flour (wheat or other) and 2.5 ml (1/2 teaspoon) of yeast. Add 750 ml of tap water to each bottle, cap, and shake vigorously. Place the 22 deg C bottles at room temperature and the 4 deg bottles in a refrigerator. Use thermometers to check the temperature of the room and refrigerator. Adjust thermostats or labels as needed.

1. The effect of sugar concentration of the rate of ethanol production.

Obtain eight 1-liter plastic soda bottles. Label the bottles 0 ml, 25 ml, 50 ml, 100 ml, 150 ml, 300 ml, 400 ml, and 500 ml. Add 15 ml (tablespoon) of whole-grain flour (wheat or other) and 2.5 ml (1/2 teaspoon) of yeast. Add 750 ml of tap water to the 0 ml sugar bottle, and add water to the same height as that one in the remaining bottles. This adjusts for the different volumes of sugar. Cap and shake vigorously. Place the bottles at room temperature (22 deg C).

Although you used the volume of the sugar to prepare the solutions, it would be good to express the sugar as concentrations of mass per volume of water. To convert the volume of sugar to mass, one could simply weigh the sugar, or use the known specific gravity of granulated sugar to calculate the mass. Water has a specific gravity of 1 by definition (at room temperature and sea level). Granulated sucrose has a specific gravity of 0.849. So to convert volume in ml to mass in grams, multiply the volume of sugar by 0.849.

Converting sugar to concentrations

|  |  |  |
| --- | --- | --- |
| Sugar volume | Sugar mass (volume times 0.849) | Concentration of sugar  (mass g /750 ml) |
| 0 | 0 | 0 |
| 25 | 21.2 | 0.028 |
| 50 |  |  |
| 100 |  |  |
| 150 |  |  |
| 300 |  |  |
| 400 |  |  |
| 500 |  |  |

Taking measurements and observations

Use your sense of smell to determine if ethanol is present. Then measure the alcohol content using a hygrometer. Pour the fluid into a beaker or jar and float the hygrometer. Read the density and convert to ethanol content.

Data table Experiment #1

|  |  |  |  |
| --- | --- | --- | --- |
|  | Smell alcohol? | Density | Alcohol content % |
| Yeast A |  |  |  |
| Yeast B |  |  |  |
| No-Yeast A |  |  |  |
| No-Yeast B |  |  |  |

Data table Experiment #2

|  |  |  |  |
| --- | --- | --- | --- |
|  | Smell alcohol? | Density | Alcohol content % |
| Grain A |  |  |  |
| Grain B |  |  |  |
| No-Grain A |  |  |  |
| No-Grain B |  |  |  |

Data table Experiment #3

|  |  |  |  |
| --- | --- | --- | --- |
| Temp. in Deg C | Smell alcohol? | Density | Alcohol content % |
| 4 A |  |  |  |
| 4 B |  |  |  |
| 22 A |  |  |  |
| 22 B |  |  |  |

Data table Experiment #3

|  |  |  |  |
| --- | --- | --- | --- |
| Sugar concentration | Smell alcohol? | Density | Alcohol content % |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

Questions

1. Use a bar graph to display the alcohol concentration data from Experiment #1. Plot the average of the A and B replicates of each treatment. What can you conclude about the role of yeast in fermentation? What job does it do?
2. Use a bar graph to display the alcohol concentration data from Experiment #2. Plot the average of the A and B replicates of each treatment. What can you conclude about the role of grain in fermentation? What does the grain contribute to the process?
3. Use a bar graph to display the alcohol concentration data from Experiment #2. Plot the average of the A and B replicates of each treatment. What can you conclude about the role of temperature in fermentation?
4. For experiments 1–3 we used replicates of each treatment. Why did we bother to use replicates?
5. For Experiment #4, plot on the X-axis the concentration of sugar and on the Y-axis the alcohol concentration. Note that the X-axis sugar concentration values will not be evenly spaced. Choose the scatter-graph option. Explain the shape of the curve. Does adding more sugar always lead to more ethanol production, or is there a maximum level? Explain.

Use a hygrometer to measure the alcohol content in each brew.

**Questions:**

1. Did the gas from the methane digester ignite? What does that tell you?

2. Was there an odor associated with the methane digester gas? What did it smell like? What is the source of that odor?

3. What happened to the indentations on the side of the sugar fermentation bottle? What does that tell you?

4. What odor did you notice from the sugar fermentation reactor?

5. What role did yeast play in the production of alcohol?

6. Compare the two processes. Consider the differences in feedstocks used by each, and how the products of each process can be put to good use. Now think broader. Which of these processes can also be put to good use to help eliminate an important type of pollution?

7. What roles does temperature play in determining the rate of alcohol production? Was there a difference in alcohol content at the two different temperatures?

8. Did the bottles show evidence of pressure buildup? What was the gas that caused that pressure?

9. Discuss the next steps needed to isolate and make the ethanol useful as a biofuel.

**Digestion of manure experiment**

1. Obtain four plastic 1-liter soda bottles. Label two bottles 22 deg C, and the two others 4 deg C. Add 1,000 ml 4 cups) of kitchen waste to a blender. Add enough tap water to homogenize the material. Add 250 ml of the homogenate.

Data table Experiment #1

|  |  |  |
| --- | --- | --- |
|  | Smells like? | Evidence of pressure? |
| 4 A |  |  |
| 4 B |  |  |
| 22 A |  |  |
| 22 B |  |  |

**Questions**

1. What can you conclude about the role of temperature in the role of digestion of manure?
2. What gases were produced? How do you know?
3. Discuss the next steps needed to isolate the methane to make it available to use as a biofuel.