EXPERIMENT 4: Methane production from food waste and garbage

Objectives:

Learn about methanogens

Understand the chemistry behind the production of methane from food waste

Identify the gas or gases produced during the reaction

Background:

Biogas is 60-80% methane and is created by a process termed anaerobic digestion, leaving behind a nutrient- rich substance termed digestate. Anaerobic digestion is carried out by a range of bacteria in the absence of oxygen. Initially carbon dioxide is produced aerobically by the decomposing organic matter until an anaerobic environment is created. After the initial digestion a group of bacteria known as methanogens convert the feedstock into methane and carbon dioxide.

Anaerobic digestion has a number of environmental benefits including production of 'green' energy and natural fertilizers. The process of converting organic feedstock into biogas can can serve as a substitute for fossil fuels and artificial fertilizers, reducing the amount of greenhouse gases released into the atmosphere. The problems associated with waste disposal are also alleviated by the generation of useful products and decreased release of the potent greenhouse gas, methane, from landfill sites.

Methanogens are obligate anaerobes that cannot grow in the presence of oxygen. They use CO_2 as the final electron acceptor and Hydrogen as a source of electrons. The reduction of CO_2 produces methane gas as a byproduct of cellular metabolism. Methanogens are abundant in swamps and sludge water. They play an important role in biomass degradation and CO_2 consumption.

$$CO_2 + 4\ H_2 \qquad CH_4 + 2\ H_2O$$

Methane is a colorless and odorless gas and the main component of natural gas (over 75%). Methane is combustible and is a major source of fuel for heat, cooking and electricity production. Natural gas is a fossil fuel that is not renewable. Although there are still huge deposits of natural gas in the US, extracting them is expensive and is environmentally destructive, and finding alternative renewable sources of methane (biogas) is a high priority.

Methane burning consumes oxygen and produces carbon dioxide and water.

$$CH_4 + 2O_2 \qquad CO_2 + 2\ H_2O$$

Methanogens are abundant in anaerobic freshwater such as swamps, the stomachs of ruminants, and sewage sludge. Methane is produced naturally by the anaerobic decomposition of organic matter. (Think swamp gas.) Finding new organisms that can efficiently convert biomass into methane is an active area of research.

Using food waste and garbage to produce methane appears to be an ideal way to use biology to solve two societal problems - to produce fuel while getting rid of garbage.

In this experiment food waste is incubated with sludge water in a tightly sealed container. Aerobic decomposition occurs first and CO_2 is produced while Oxygen is consumed. Once the oxygen has been depleted the methanogenic bacteria start growing and consume the CO_2 to produce methane.
Therefore, one should expect an initial increase in CO_2 production followed by a decrease in CO_2 and a steady increase in methane production. The biogas obtained at the end of the reaction will be a mixture of CO_2 and CH_4 . The CO_2 can be monitored using a CO_2 detection probe. The methane can be identified by burning it. The biogas will be collected in a balloon and burned under the hood.

Materials:
Glass bottles with stoppers (we will use 125 ml serum bottles.) You can also use plastic bottles with outlets
Needles (18 to 22 gage)
Blender
Tap Water
Sludge or swamp water
Graduate cylinders
Tubing
Balloon

Procedure:

- Fill half of the blender with food waste from your kitchen.
- Add water
- Turn on the blender. Add enough water to obtain a mixture that has the consistency of a hearty soup: thinner than chowder but thicker than bisque.
- Add 40 ml of the waste mixture two each of 250 ml BOD shaped bottles.
- Label one flask: Sludge and the other water (control)
- Use a graduate cylinder to add 10 ml of bacterial sludge (now 20% by volume of the total) from Deer Island or other sources to the flask labeled sludge.
- Add the same amount of tap water or autoclaved sludge to the other control flask.
- Check the pH. It should be around 7. If necessary add some base to increase pH to 7.
- Seal the bottle with a blue stopper (Wet the stopper first to make it easier to push into the bottle).

- Next, take a new 1-ml insulin syringe and remove the plunger, cut the top of the barrel to remove the flaps. Then screw the bottom of the syringe into the needle.
- Attach the top of the syringe barrel to a length of soft (tygon) tubing – it should be airtight. If necessary, use electrical tape to seal the connection.
- Attach a balloon to the end of the tubing OR bubble gas into a collecting tube over water
- When the balloon-tubing-syringe-needle apparatus is complete, you may then carefully insert a needle into the stopper and push through until it comes out the other side of the blue stopper.
- Incubate the set-up at 37ºC.
- Measure CO_2 produced using the method you have chosen.
- At the end of the experiment check the pH of the solution.
- Add bleach to liquid waste and discard
- Throw solid waste into burn box.

If time permits you can dry and weight the solid waste

Question:

What gases are produced?
How could you measure the amount of CO_2 and CH_4 produced?
What are the limiting factors in the production of CH_4?
How could you increase methane production?
What are the economical and ecological implications of biogas production?
How can pH influence methane production?

In this activity you will design a biogas generator from simple material, collect the gas produced, and test it by using a Bunsen burner to burn it

Designing and constructing a biogas generator makes an ideal project for students to express their creativity and problem-solving skills. The format of this exercise should be adjusted to suit the circumstances of the class and time available. It can be carried out in an hour-long session if sufficient materials are provided for the students to construct biogas generators and the gas from an already established generator is tested. This activity works well as a long-term project that can be revisited periodically with a school or class. It can take up to six weeks to produce enough biogas to burn.

What you will need

- Water cooler bottle or fizzy drinks bottles
- Rubber tubing
- Clamps
- Bung or bottle top

•Measuring cylinder
•Tape
•Plastic tubes (a biro can be used so long as the hole in the tube is covered with tape)
•Mylar/foil balloon (rubber balloons are porous and allow the gas to escape)
•A variety of organic matter such as grass clippings, leaves, waste fruit and vegetables, tea bags
•Bunsen burner and heatproof mat
•Plasticine or blue-tack
•Disposable nitrile gloves

EXPERIMENT 4A: isolation of methanogens from soil and water samples

Transfer 50 µl of sludge or swamp water onto an LB agar plate. Using a long sterile wooden stick or glass rod, streak or spread the water onto the plate.
Seal and incubate in a chamber with normal or low O_2.