Construct and test a bench scale anaerobic digester.

Safety

• Students will be wear apron, goggles and gloves for the experiment.
• Students will be instructed in the safe handling of effluent.
• Students will add acetic acid solution to bottle of effluent in the fume hood or in a well-ventilated area.
• Students will transfer the mixture to the water bottle in a fume hood or well-ventilated area.
• Students will be instructed in the proper procedures for use of anti-bacterial soap in cleaning up effluent spills and for disinfecting lab benches at the end of each lab period.

Materials:

For each group of 2-3 students:

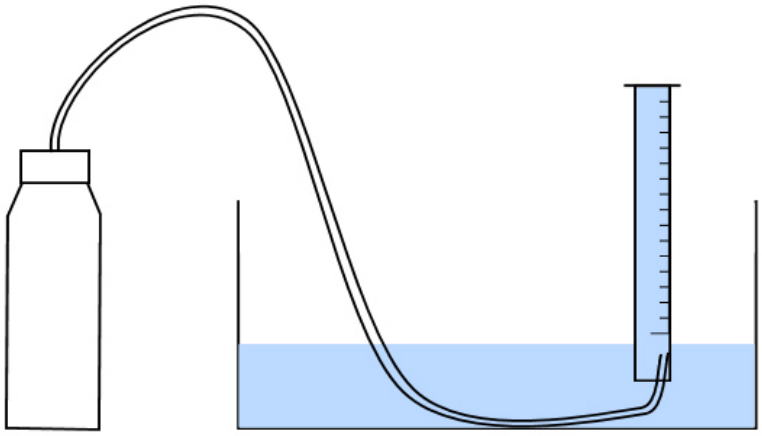
2 -water bottle with sports cap with 125 ml of anaerobic effluent added
500 ml Erlenmeyer flask with cork
Funnel
Vinegar (acetic acid)
Baking soda
pH paper
Matches or lighter
Goggles, apron and gloves
Tubing and pinch clamp

(Note to instructor: secure fresh sludge (microbial inoculum) from an anaerobic digester such as Quasar or a local water treatment facility. The sludge should be stored at 4 ˚C if it is not used immediately. Care and clean work practices should be exercised since you are working with a microbial substance.)

Procedures:

1. Obtain materials from instructor using goggles apron and gloves,.
2. For each 500 ml water bottle or sports bottle, dilute 24 ml of acetic acid with 50 ml of tap water and add sodium bicarbonate to adjust pH to neutral, about 2.6 grams. (Note other biomass feedstocks can be used instead of vinegar. However it is important to maintain a neutral pH. To save time make double the amount and put half into each of the two bottles.)
3. In a fume hood, add the effluent to the Erlenmeyer flask containing the vinegar (or other) solution. Add tap water to bring the total volume up to 500 ml. Cork and mix thoroughly.
4. Using a funnel, add the mixture back into each of the water bottles.
5. Over a sink, carefully and slowly, squeeze all of the headspace out of the bottle until the liquid level reaches the bottle top. Place closable lid on bottle and lock the bottle cap. (Note: these two steps insure an anaerobic condition inside the water bottle, which is absolutely critical.)
6. Place tubing on bottle with a pinch clamp clamped close to the opening of the cap.

7. Incubate the bottle in a warm place for anaerobic fermentation to proceed. (Note: the ideal temperature for anaerobic digestion is between 35 and 40 ^{O}C, at which the bottle would be filled up with biogas in several days; at room temperature it may take weeks.)
8. In the course of fermentation, biogas can be monitored with a simple water displacement method as shown:



9. Each day, at the same time, using the apparatus shown, open the pinch clamp, then the cap to release the gas and displace the water. Close the pinch clamp and cap quickly when the water has been displaced and record the volume of water displaced in a table. Return the bottle to incubation until the next day's readings.
10. After 4-7 days (longer if kept at room temperature) end the test and confirm that methane is present in the bottle. Take the hose and place it at the bottom of a large beaker of soapy water. Open the cap and bubble the gas through the water. Then take a match or flame and carefully try to light the bubbles.
11. Record the volume of gas each day and for each day, add the total gas collected up to that day. Graph the amount of gas collect versus time. What does the graph show you?
12. Verify the presence of methane by bubbling some of the gas through soapy water and carefully lighting with a lighter. Are there other gases that evolve? What might they be?

Resources:
Anaerobic Digestion: http://www.youtube.com/watch?v=UzD53yBbjyI