Abstract

This report chronicles the investigation of the different factors affecting household scale and minor industrial applications for anaerobic decomposition, focusing on the affects of temperature, pH, and nitrogen / phosphorous levels on the production of biogas and methanogens.

The first experiment and investigation was done in 2003, focusing on differences in biogas production between heated and non-heated anaerobic reactors. This experiment simply highlighted the known information that heat speeds up the reaction of the organic waste, but also confirmed that for a household reactor biogas generation was possible.

The second experiment was undertaken in 2004, after the temperature levels had already been established, was to take a more in depth look at the biology of biogas production through the effects of pH on methanogens. Using an understanding that methane producing bacteria reproduce and live at a pH of 6-7.5 (neutral-slightly alkaline), and at an optimal temperature of 35-38.5 °C, two reactors that would be able to insulate and heat the slurry to the above temperature, and apparatus to measure the pH were designed. Reactor 1 was pH adjusted, to an average of 7.06. With the combination of a neutral pH, a wet environment, and a high mesophilic temperature, it produced 64mL of Biogas (over 70% methane) over 47 days. The other Reactor, with no pH adjustment, produced no biogas in that period. The experiment created an environment where methane producing bacteria, and aceto-bacters (acid making bacteria) could thrive together (the acid produced by the aceto-bacters is eaten by the methanogens to produce methane). While the aceto-bacters produced acid, the methanogens, which are very sensitive to temperature and pH, could eat the acid and produce methane. The experiment overall created a false environment, which does not usually occur in nature for both types of bacteria to reproduce at the same time. After finding how the process of biogas production from a household could be improved or optimized at a smaller cost by creating a false environment, the next step was to see if the idea of optimized artificial surrounding could be applied to the growth of the actual methane producing organism’s methanogens.

In the third experiment conducted in 2005, the use of adding nitrogen and phosphorus rich compounds to enhance the growth and development of methanogens was explored to see its effects. The focus was on the growth of target bacteria required for anaerobic decomposition or composting, by manipulating the chemical environment within the feedstock towards the goal of accelerating microbial production. This was accomplished by sampling partially completed compost slurries and measuring bacterial counts microscopically. The key variables studied were the C: N (Carbon to Nitrogen mass ratio) and C: P (Carbon to Phosphorous) ratios, relative to unmodified control slurry. In order to maximize microbial growth in small (1 L) model reactors, the feedstock surface area was maximized by creating a wet slurry (using a blending device) from a base sample of kitchen and yard waste. By utilizing optimal conditions for temperature (in the mesophilic range), starting pH, and feedstock preparation, a comparison in the number of methanogenic bacteria (since the test was conducted under anaerobic conditions ) was made by sampling over the course of a 23 day reaction period (two repetitions per condition). A difference in the number of bacteria was observed between the two test reactors, and relative to the control reactor environment. Based on the small sample size and only two repetitions, one can conclude that there is a trend suggested, but it is not statistically significant on this base size. Further work will be focused on longer and more trials at the test conditions, so as to provide a statistically significant basis to make the interventions during waste collection (blending of Nitrogen, Phosphorous and Carbon containing wastes) to maximize bacterial production, and ultimately reach optimal biogas production conditions. Also explored throughout the periods of investigation and experimentation was how this knowledge could be applied to a more large scale industrial process. In the industrial process the period of settling or digestion would be prime for the addition of a compounds geared towards controlling the pH and growth of bacteria, to make the use of anaerobic digestion
Experimental Research

Overview of Composting

Composting is a method of solid waste management where the organic component of the solid waste is biologically decomposed under controlled circumstances to a state where it can be hauled, stored, and/or applied to the land without adversely affecting the environment. By products of the decomposition are gases and/or water vapour (depending upon the conditions).

The most key phrase in that definition is under controlled circumstances, because it distinguishes composting as a biological decomposition process responsible for the recycling of nutrient elements in nature.

There are 3 main comparisons of composting classification:

1. aerobic vs. anaerobic
2. mesophilic vs. thermophilic
3. mechanical vs. non-mechanized

These three terms have practical significance in the aeration of the compost, the temperature of the compost, and the technological aspects of the make of the compost storage container or the organic component is organized. The term organic component follows from the condition of biological decomposition. In general only matter of biological origin is directly subject to biological degradation.

There are 2 types composting: - aerobic: bio. decomposition process with the presence of oxygen - anaerobic: bio. decomposition process in the absence of oxygen

The biology of Aerobic Digestion

Aerobic Digestion- Aerobic Digestion is a process of decomposition in the presence of oxygen, generating carbon dioxide, and nutrient rich soil. It is the opposite of Anaerobic Digestion. It can be run at thermophilic or mesophilic temperatures.
Aerobic Compost contains 5-10% living organisms. Some of these include worms, and various bugs. They are vital to the aerobic process, by releasing key nutrients that need to be broken down. The following Chart shows the percentages:

**Biology of Anaerobic Digestion**

**Anaerobic Digestion**- A biological process, that produces a gas principally composed of Biogas (methane carbon dioxide and other gases), in the absence of oxygen. These gases are produced from organic wastes (livestock manure, food wastes, etc.).

So called ‘mass balance’ of a model anaerobic digestion process:
**Anaerobic Digestion** is a type of composting that is not generally used in the household for decomposing solid organic matter. The aerobic process is used instead because the main objective of most people who compost at home is to reduce garbage and make compost to be added to the soil. As well, not many people have access to air-tight containers in which to collect the biogas, or a way to use it, under anaerobic conditions.

Anaerobic decomposition can occur naturally or under controlled environments such as in a digester. The gas produced via the biological reactions can be over 95% methane, which could be harnessed, while reducing the amount of waste. In the literature, there are 4 sets of conditions that distinguish different types of processes:

1. **Solids content** – at 10 to 15% solids, it is referred to as ‘wet compost’
   - at 20 to 40% solids, it is referred to as ‘dry compost’

2. **Temperature**: at an operating temperature averaging 35 degrees C – mesophilic
   at an operating temperature averaging 50 degrees C – thermophilic

**Description of the process (Overall)**: With a feedstock of organic materials, and water, during digestion a fraction of the solids are converted to biogas due to biochemical reactions. There is also a small amount of Hydrogen sulphide produced (H2S), but it is so low, that it seems to help conversion. At the end of process, the digester residue, waste water (including some water vapour) and non-recyclables remain. The residue is made up of volatile solids not reacted, microbial biomass, and some fraction of solids not converted due to incomplete reaction. The biomass is useful in agriculture.

**Comparing Wet and Dry systems** in Wet systems, the organic solids are less than 15% of the weight of the reactor loading, the balance being liquid. Because of the moisture, usually a continually stirred type of reactor set-up is needed. It is more expensive to run than dry systems, and there is work to keep the liquid mixed and heated and more difficulty disposing of solids at the end (not to mention energy requirement to dry the liquid). This approach is used offer for sewage sludge or other industrial wastes, and can operate in either mesophilic or thermophilic temperature. In Dry systems, the solid content is up to 40%, and requires less mixing, and is easier to dispose of the resulting biomass. It also can be run under thermophilic or mesophilic temperatures.

**Comparing Mesophilic and Thermophilic** –

Mesophilic decomposition is a type of aerobic/anaerobic digestion that operates around 35 degrees C. Any higher than 40 degrees C and these bacteria begin to die off. They are replaced by thermophilic bacteria. Mesophilic anaerobic digestion is cheaper in heating costs than thermophilic, because less energy for heating is required. At higher than 65 degrees C, most bacterial are destroyed. This is why operators of anaerobic digesters stir up the reactor tanks so that wet slurry does not get too hot.

In a study done by the Institute of Water & Environment of Cranfield University in 2002, about the effectiveness of the two types, reached the following conclusions:
- the mesophilic digester achieved greater solids destruction (reaction) and better
dewatering characteristics (less need for drying). This has an impact on storage and
transportation costs of the resulting biomass.
- The thermophilic anaerobic digester effectively pasteurized the reactant (killed all the
organisms). This is helpful where regulations require sludge from compost needs to
be pasteurized before application on agricultural soil, such as in Switzerland.
- The thermophilic aerobic digester incurs a higher energy cost to run, since the large
pumps needed to mix or circulate the sludge, and to heaters to keep it at temperature.
This would be the main reason they are not competitive with mesophilic systems.
- - the mesophilic systems, operating at lower temperature, require less energy, and can
use the biogas produced.

In summary, the conclusions of the scientists were that mesophilic digestion was preferred,
particularly for small scale (non- sewage sludge) applications.

### On pH and Anaerobic Decomposition Stages

In anaerobic decomposition, there are three main stages. The first stage is the
breakdown feedstock into small usable molecules such as natural sugars, amino acids, and
fatty acids (basic building blocks), by enzymes.

The second stage is the acidification of molecules that weren’t fully broken down by
the enzymes, such as protein, carbohydrates, fats, and cellulose. These molecules are made
into simple compounds of O$_2$ and H$_2$ called volatile acids. This part of the decomposition
process is done by microorganisms called aceto-bacters. The acid produced is acetic acid.
The process of the pH dropping is called souring. The pH falls under 6, which is below the
pH at which methanogens (methane producing) microorganisms cannot live, or reproduce.

The third stage is the transformation of the acids and basic compounds into methane
(biogas). The methanogenic microorganisms are very sensitive, and do not reproduce
quickly. They thrive in a moist environment, and can live in over 60% water content. The
bacteria also reproduce well in a neutral to slightly alkaline pH (6-7.5, or some times the
research will say 7-8.5, but only occasionally). The biogas produced is over 75% methane,
with 20% CO$_2$, and the other 5% consists mainly of H$_2$. I have made an equation, which
shows these stages.

$$\text{Organic Matter} + \text{H}_2\text{O} + \text{Nutrients} + \text{Aceto-bacters} - \text{O}_2 = \text{Methanogens} +$$
$$\text{Heat} + \text{H}_2\text{O} + 6-7.5 \text{ pH} = \text{CH}_4, \text{ CO}_2, \text{ H}_2 = \text{Organic compounds stabilized}$$

### The Bacterial Process:
Some of the microbes that thrive in composting can operate in either anaerobic or aerobic
conditions, although the population mix (types) differs (some more likely at higher vs. lower
oxygen content). Bacteria - studying he bacteria in both thermophilic and mesophilic
digestion is quite interesting. Bacteria are the smallest living organisms, and make up about
80 to 90 percent of the billions of microorganisms living in a single gram of compost. They
use a variety of chemicals called enzymes to break down the organic material (containing
carbon). The bacteria themselves are single-celled and structured either rod shaped bacilli, sphere-shaped cocci or spiral shaped spirilla. They are ‘motile’, that is they can move. There are many types of bacteria that survive and thrive in different conditions. Most of the bacteria that are prolific in an anaerobic digester are ‘ancient’ bacteria – those that can survive in no or low oxygen conditions.

At the beginning of the digestion process (up to 40 degrees C) mesophilic type of bacteria are the most prolific. These types are typically found in topsoil. At temperatures above 40 degrees C, the thermophilic bacteria take over. These are typically the rod-shaped bacilli. The diversity or numbers of types of bacilli are quite high at temperatures from 50 to 55 degrees, but they begin to die off at 60 degrees quite quickly. When conditions are unfavourable, the bacteria form endospores, thick spores that resist heat and dryness, but also cold and lack of food. They become active again as the temperature drops. At very high temperatures, the bacterium that dominates is of the type Thermus. These are less plentiful, but manage to survive. They were first identified in the hot springs of Yellowstone National Park. As the temperature of the biomass drops, the bacteria most active at that temperature range take over.

Another class of micro organisms in compost are fungi, which include moulds, yeasts, and these can break down quite complex organic polymers, which the bacteria cannot. Cellulose is one example. Fungi grow rapidly spreading extensions of themselves, to break down tough debris. They also attach organic residues left by the bacteria. They are plentiful in both thermophilic and mesophilic stages. Compost moulds are aerobes that can grow as invisible filaments or greyish-white fuzzy mould on the surface of the compost or slurry (as seen in the control reactor at the end of Trial 2 and a small amount in Trial 1 of my experiment).

Actimonycetes are interesting class of bacteria in the anaerobic and aerobic mesophilic and thermophilic composting. They are important in the early stages of the anaerobic compost. This micro organism causes the characteristic earthy smell of soil. This bacterium resembles the look of fungi bacteria, but is actually filament like bacteria. Like bacteria they don’t have a nuclei, instead they have multicellular filaments like fungi. In the digestion process they are the bacteria that have the important act of degrading complex organics like cellulose, and proteins. Also, their tough enzymes break down debris like woody stems, bark, and newspaper. Actimonycetes form threadlike filaments stretching through compost and sometimes appear in a circular shape that expands over time.

**Methanogenic Bacteria Mechanisms**

While the family of bacteria is important, the actual conversion of acids and carbon dioxide to CH$_4$ is undertaken by archaea (ancient bacteria) called methanogens, also referred to as methanogenic bacteria. These ancient (they were one of the first micro organisms on the planet) organisms are responsible for the conversion of organic acids to biogas, and are the only organisms that produce methane. Methanogens, which range from 0.4 micrometers to 1.7 micrometers length in size, come in many variations of shape like other bacteria. There are three types of substrates (feedstock within the compost slurry) that methanogens use to convert to CH$_4$. The three substrates are known as:
- CO₂ type (using CO₂ and H₂ from their environment and food to make CH₄ and H₂O);

- Methyl type (converts methanol and hydrogen to CH₄ and H₂O) and;

- Acetate (a type of cellulose combined with again H₂ to produce CH₄.

The majority of methanogens use the CO₂ substrate. Methanogens while being the most important part of the digestion process are very fragile, and must have a neutral to slightly alkaline pH (6-7.5), and temperatures in the mesophilic or thermophilic temperature range. Otherwise, the methanogens will die (in the case of above thermophilic temperatures) or reproduction will stop (while not out of the mesophilic temperature range, an average reactor temperature of above 30°C is optimal). By being so particular and also the most prolific organisms at converting waste to biogas, become the controlling factor in establishing the physical and chemical environment in the system. Below are two images of methanogens – Figure 1 is an electron microscope image of the outside of the bacteria. The second, Figure 2, shows how they may appear sampled using an optical microscope.

Affects of Nitrogen and Phosphorus

In anaerobic digestion, many nutrients affect the methanogens reproduction and gas production. Two of the major nutrients are nitrogen and phosphorus. Phosphorus like nitrogen is also required for cellular reproduction. During the methanogenesis stage (this is when the bulk of the organic acids are consumed by the methanogens and made in to CH₄) of digestion, nitrogen and phosphorus are needed to promote methanogen growth (therefore continuing biogas production).

The carbon to nitrogen ratio in anaerobic digestion is important because it controls biogas production, and that to even start the process of reproduction nitrogen is needed. The C: N ratio can be controlled by blending feedstocks. There are many sources of information concerning the C: N ratio. The wide variety of ratios is at times contradictory. One source says that the optimal ratio is 20:1 (www.lsuagcenter.com/Communications/pdfs_bak/compost), while others say that as high as 40:1 is reasonable (Pace Michael G., The Composting Process, Utah State University, October 1995). Even so the majority of sources say a C: N ratio of 20-30:1 is optimum.
(sources ranging from basic composting guides to university studies), because if the C: N ratio in the digestion process is too high, it allows the accumulation of ammonia which destroys the bacteria, and if the ratio is too low it limits growth and reproduction of the bacteria. To provide a medium amount of nitrogen in the digestion process a ratio of 25:1 would seem optimum because it is in the middle of what is considered the best range. There is a small enough amount of nitrogen as not to border on destroying the bacteria with excess ammonia, nor possibly under calculating the amount and slowing the bacterial metabolic and reproductive processes. By having a balanced C:N ratio, methanogen and general bacterial production will thrive. The C: N ratio can be controlled by blending feedstocks to adjust the ratio (sampling to determine the biologically available carbon and nitrogen could be undertaken), or in a laboratory environment, by ‘doping’ the system with specific high nitrogen ingredients or carbon containing materials.

The carbon to phosphorus ratio is less discussed than the carbon to nitrogen ratio, because added phosphorus serves as a booster rather than deciding factor when added to a reactor environment. While not as discussed as C:N, C:P ratios are all very low such as 100:0.08 (Creed 2002, Veolia Environment Data Sheet “Principle of Waste Decomposition”, 3p), or 150:1 (On Farm Composting Methods, Food and Agriculture Organization of the United Nations). In anaerobic decomposition, added phosphorus is generally low because there is so much of it already present, even in the decomposition of kitchen and garden waste (most anaerobic digestion processes use manure as feedstock). By adding phosphorus (pure) to a reactor’s slurry, it acts a supercharger to bacterial reproductive and metabolic systems making them produce waste waster (in this case the byproducts being biogas in the case of methanogens and organic acids in the case of aceto-bacters).

Through the manipulation of phosphorus and nitrogen in anaerobic decomposition a perfect amount of fertilization could be achieved, making sure that methanogens have the proper mix to thrive in the reactor environment and improve reproduction rates and therefore biogas. While a simplified diagram of elemental nitrogen is shown below, as well as phosphorous in the form of red modified phosphorus (a type of phosphorus that will not ignite in the air), the key complexes utilized by methanogens are nitrate (NO$_3$-N) and phosphoric acid (P$_2$O$_5$).

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**Industrial Scale Anaerobic Digestion**

Andrew Darby 8
In the industry of composting, many plants/reactors use the anaerobic process of decomposition, mostly because the biogas generated is valuable. The rich humus is an added bonus. Also, at the industrial scale companies find it more profitable to use animal manure ranging from chicken to cow, instead of organic waste, such as yard and kitchen. A proven anaerobic digestion process is the BTA Process, in which the material can be taken straight from the landfill and separated into organics, or contaminants (plastic, glass, and metals). This “hydro pulping” produces an organic pulp. This is the first patented step. The second patented step is to use a de-gritting system in which any glass, small stones, or sand. The left over organic pulp is then anaerobically digested either in the thermophilic or mesophilic temperature ranges. This process produces 2 high value products, biogas for power and electricity for the plant and power plants, and anaerobic compost to be spread on to the land as humus. Any un-digested matter is inert, and doesn’t have any active biological elements in it. This process takes just a couple days, and because it is fully enclosed and computer controlled it is void of any health problems. This process, which originated in Germany, is also used by Canada composting Inc. These plants run on household organic waste, unlike others that are on manure. Around the world there are over 650 anaerobic plants worldwide producing nutrient rich humus, and the main resource/product, the valuable biogas.

The biogas can be used for cars, once properly refined, and it can, and has, been used to power electric generators, to make it self-sufficient. Also, because it is combustible, it can be used in major electrical plants. The decomposed organic matter can be used in farmers’ fields to fertilize the land, by adding rich no-bacteria humus to the soil, giving a higher crop output.

Since it is natural gas, all gas-run inventions can utilize it, such as for home heating, and barbeque tanks. In Europe there are large industrial scale digesters for these purposes. One Anaerobic digestion facility treating 100,000 tonnes would produce enough biogas to power and light 8,000 homes each year; and would produce enough compost to cover over 24 Subiaco Ovals to a depth of 10 cm (according to the authors). There are numerous industrial Applications for this research experiment, and at home.

At the household scale the biogas can be used to run small electrical generators for emergency use or for a lowered electric bill. Since I have proved that pH adjustment can work on household reactors, it could mean a whole new view in composting, where the process output could increase enough that on a household scale the composting process could be a practical way to produce energy.

**Experiments**
To highlight the research, two experiments taking place over two years (2004-2005) are provided. The first experiment taking place in 2003 was a preliminary test to see if biogas generation was possible at home in a small reactor. The second and third experiments deal more with the biology and how the generation of biogas can be applied in an industrial setting, through use of chemical compounds to affect the growth of methane producing microbes.

**Experiment #1 (2004)**

**Hypothesis:** The anaerobic digester with the pH adjustment will produce more biogas (over 70% CH4) than the unregulated one because methane-producing bacteria thrive at temperatures of 35-38.5°C, and at a near neutral or slightly alkaline pH of 6-7.5.

**Purpose:** The purpose of the project was to see if a heated household scale model anaerobic reactor, using kitchen and yard waste feedstock, with a pH adjustment to the neutral or slightly alkaline pH range (6-7.5) would produce more biogas (over 70% CH4) than one, which had no pH adjustment.

**Variables:**
- **Independent Variables:** The pH of Reactor 2
- **Dependant Variables:** The amount of biogas generated by the digesters/reactors, over a 45-day period, and the pH of Reactor 1.
- **Controlled Variables:** Were size of reactor design, moisture content, and feedstock.

**Procedure (Overall)**

In this experiment, two airtight anaerobic digesters heated in the mesophilic temperature range were set up. One of the containers (Reactor 1) had sodium bicarbonate added to raise the pH level to 6-7.5. The other digester (Reactor 2) was left to decompose without any unnaturally occurring pH changes. Two airtight containers, two beakers, two graduated cylinders, one aquarium, and tubing were used to make both digesters/reactors. I then filled the reactors with a kitchen scraps/leaves/dirt mixture (which had been conditioned in container for approximately 1 month), took a pH sample and then continued every week, and took temperature every day for 45 days.

**Preparing and separating the slurry**
- Collected kitchen scraps which included coffee grounds and eggshells in a bucket (I also used some of last years mix. I tested the pH of the mixture in three different areas (6, 5.5, 5.7, average=5.73)
- The mixture was added to until January 25th, and then dirt leaves and water was added for a good mixture of diet, nitrogen, and earth: 2L of dirt
  - 1.5L of leaves
  - 1.8L of water was mixed in.
- The slurry was then separated into two equal parts (2.7L) using the fill volume of the reactors/digesters (the calculation is in the Appendix A).
- The mixture was put into the two reactors/digesters. The pH was tested in both reactors/digesters it was 4.6 for both. In Reactor 1, 125g of sodium bicarbonate (baking soda) were mixed in to raise the pH of the slurry to the range of 6-7.5. When the sodium bicarbonate was mixed in the pH jumped to 6.5. No baking soda was added to Reactor 2.

Andrew Darby

1
Modifying/building the reactors/digesters

1) The materials required to build the reactor/ digester were collected.
2) Duct tape was used to secure 1947.35 cm² of reflective insulation around the outside of the aquarium.
3) 651.2 cm² of fibreglass insulation was put in the bottom of the aquarium and the reflective insulation was used to cover it.
4) Silicon was used to secure a 20cm*20cm piece of wood into the aquarium, to divide it by its width.
5) Two identical 100-watt new aquarium heaters were attached to the aquarium, one on each side of the wood.
6) The work light, which had a 60-watt incandescent bulb, was attached to the aquarium.
7) Last year’s reactor was taken and 20 cm of 3/8 inch diameter tubing was attached, then a T-valve to it. Two 2in pieces of plastic tubing were attached, each to its own valve. One of the valves connected to a 500mL flask with a 20 cm piece of tubing, which then connected to a 250mL-graduated cylinder. The other valve is used to insert sodium bicarbonate and vinegar (Reactor 1 only)
8) A hole was drilled into the lid and inserted 10cm of tubing, then a valve and the more tubing was added so slurry could be collected for pH testing.
9) 10 holes were drilled in a copper tube 1.5 cm diameter, and had a stand made out of 0.5 cm thick solid copper tubing. The purpose of this was to collect CH₄ from pockets inside the slurry as landfills do.
10) These steps were repeated for Reactor 2.

The experimental procedure

4. The fill volume of the reactors was calculated (2.7L).
5. In the reactors, the copper tubes were placed.
6. The reactors were filled with 2.7L each of the slurry.
7. The temperature was checked everyday.
8. The pH was checked every second day, but was changed to once every seven days in case of any possible oxygen exposure during the pH testing.

Note: Last year’s flasks and stoppers were used, so below is the process in which they were modified:

- Bent glass tubing and prepared water displacement flasks (help from my father for bending glass with propane torch).

Also, the equation for calculating the fill volume of the reactors is available in Appendix A.

Materials

- 2x 1-gallon airtight storage jars (plastic)
- 2x 500 ml glass flasks
- 2x 250 ml. Graduated cylinders
- 2x rubber stoppers
- 2x meat thermometers
- One 5-gallon aquarium
- 2-aquarium heaters (100 watts each)
- 1 attachable work lamp
- 1 60-watt incandescent light bulb
- 4 ¼ inch brass tube connectors
- 1 16.5 by 19 cm wooden divider
- 1947.305 cm² of reflective insulation
- 140 cm of 3/8 inch plastic tubing

Andrew Darby
- 4 3/8 inch valves
- 2 ¼ valves
- 40 cm ¼ inch plastic tubing
- Various lengths of duct tape
- 160 mL of silicon cement
- 2 3/8 inch T-valves
- 4 gallons of water
- 1 35cc syringe
- 1 pH meter
- 7 mL of vinegar
- 125g of sodium bicarbonate

**Results:** Analysis was done of biogas production, pH, and temperature changes of the experiment. The electricity used (Kwh) to run the reactors were compared to the potential energy of the gas produced.

1. **Temperature** The average temperature of Reactor 1 was 37.26°C, and for Reactor 2 was 35.97°C. These were determined over 45 days of the 47 day experiment, because of temporary measurement equipment problems on two days. The graph below shows the temperature during the experiment, which shows that the system was able to maintain on average a temperature in the range of 35-38.5°C, except for once above (40°C), and once below (32°C) the range at the beginning of the experiment. The system was in temperature control.

![Comparisons of Reactor Temperature](image)

2. **pH of the slurries** The graph below shows the pH of the reactors. The average pH of Reactor 1 was 7.065, which is in the exact experimental specifications. The average pH of Reactor 2 was 6.288. On the 29th day of operation, vinegar was added, and pH lowered to 8.0, in Reactor 1.

![Comparisons of Reactor pH](image)
3. Biogas Generation  The graph below shows the amount of biogas generated by Reactor 1 (64 mL.), and none by Reactor 2. It was noted that in the tube leading from the flask to the cylinder there was what appeared to be condensed water. Because it did not reach the cylinder, it couldn’t be measured. The biogas generated was measured by a water-displacement system. The reactor 1 produced biogas of 24ml and 40ml in two events, on days 38 and 40.

![Overall Biogas Production Graph](image)

5. Energy Analysis  The measurement of the energy used to produce the methane and the theoretical energy the methane could produce (if combusted at 20°C) was compared. The amount of electricity used by Reactor 1 was 205.2 Kwh, but Reactor 1’s 64mL of biogas could only produce 6.35 *10^-4 Kwh. This process is not yet practical, but larger digesters and more insulation would be expected to improve it. The calculations for the numbers above are in Appendices 1B and 2B.

Conclusions

A Tale of Two Reactors

Based upon my observations I was able to generate more biogas (64mL.) in Reactor 1 than Reactor 2, and my pH average, and temperature average were within the pH and temperature constraints. The hypothesis therefore was proven correct that a household scale anaerobic digester with an adjusted pH would produce more biogas than a reactor with the pH left to nature.

In natural anaerobic decomposition, the slurry is broken down into amino acids, fatty acids and acetic acid, which is called souring, and the pH goes down. This slows gas production. By adjusting pH upwards in Reactor 1, in to the range of 6 to 7.5, I was able to create conditions in which both the aceto-bacters (acid producing bacteria) and the methagenic bacteria could survive and reproduce. This helps account for the 64 mL of biogas produced. However, in Reactor 2, the pH gradually rose (see chart) as the methanogenic bacteria eat the acidic components, and later it dropped perhaps due to insufficient quantities of bacteria. The drop in pH could have been caused by insufficient bacteria, relative to Reactor 1. Reactor 1 received a ‘boost’ due to the initial pH adjustment to stimulate bacterial growth.
When the pH went out of the specified range, it was all right because; I adjusted it so it would go down eventually. After I added vinegar to make the pH go down slightly, Reactor 1 produced 24mL of biogas, and as the pH dropped further, there was another push and then I had 40mL of biogas.

While it appears that in this experiment, conditions were present to allow a new way for biogas producing bacteria to thrive, I did expect some production from Reactor 2, given that the mesophilic temperature range attained should have been optimal for this size of reactor temperature range is optimal for this size of reactor. Even so, I did see condensed water, in the tube leading from the flask to the cylinder. But since it never reached the cylinder, I could not count it. Given the variable nature of the feedstock from one to another (here, they were from the same batch of feedstock), while I saw conditions favourable to gas production in Reactor 1, there may not have been enough experimental time for the unaided Reactor 2 to sufficiently breakdown the waste and produce gas.

Error Analysis

For both Reactors, acting as closed systems, it was important to keep them as airtight as possible. Using last year’s reactor design, I was satisfied that the reactors would be sufficient for the size of the system. The addition this year of more adding and measuring ports, including valves, meant there was some chance of leakage, or air entry, during sample collection. I ensured that the tubing connections were sealed (using silicon), but this could have in fact been a potential gas loss/oxygen introduction.
Appendix A

Calculation of Reactor fill (both filled equally, up 17.5 cm from the bottom)

Recall (from geometry) that the volume of a sphere is

\[ \text{Volume} = (\pi) \times (\text{radius}^2) \times (\text{height}) \] all in cm.

Here \( \text{Volume} = (\pi) \times (7^2) \times (17.5) = 2693.0134 \text{ cm}^3 \)

Or approximately 2.7 L (since 1000 cm\(^3\) = 1 liter)

Appendix 1B

Energy Analysis: Energy used

A) 60 watt light bulb = 30 watts each

\[
\frac{\text{watts} \times \text{hours used} \times \text{days used}}{1000} = \text{Kwh}
\]

\[
\frac{30 \times 24 \times 45}{1000} = 32.4 \text{ Kwh}
\]

B) 2 100-watt heaters

\[
\frac{200 \times 19.2 \times 45}{1000} = 172.8 \text{ Kwh}
\]

\[
+ \frac{32.4}{32.4} = 205.2 \text{ Kwh}
\]

Appendix 2B

Energy Analysis: Energy produced

\[ D \times V = M \]

\[ D \times 62 \text{ mL} = M \]

Below # from CRC Handbook of Chemistry and Physics, 62\(^{nd}\) edition,

\[ M = D \times V = 0.0416 \times \frac{453.5939}{16} \times 35.3145 \text{ ft}^3 \]

\[ \frac{\text{ft}^3}{16} \times 10^6 \text{ mL} \]

Therefore in 62 mL \( M = D \times V \text{ mass} = 0.000666366g \times 62\text{mL} = 0.041314g \)
Experiment #2

Problem: What is the effect of added nitrogen (in the form of urea) and phosphorous (in the form of bonemeal) on the growth of methanogenic bacteria (which generate biogas) measured independently, in model kitchen and yard waster anaerobic digesters, using a common starting slurry, operated under mesophilic conditions?

Hypothesis: I hypothesize that both the phosphorous-enriched and nitrogen-enriched digesters will have higher methanogenic bacterial counts than the control. Phosphorous is growth stimulant, and promotes reproduction of the desired bacteria. Nitrogen is a key component of the bacterial cell nucleus, and facilitates cell growth and metabolism. In addition, I hypothesize that the nitrogen-enriched reactor will out perform the phosphorous-enriched reactor, as its role in cell growth is well known.

Variables:

<table>
<thead>
<tr>
<th>Independent</th>
<th>Dependent</th>
<th>Controlled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial growth in Reactor 3 (control); final pH of slurry in all Reactors</td>
<td>Bacterial growth in Reactor 1 and 2, measured by extracting discrete slurry samples and observing microscopically</td>
<td>Reactor Design, Initial pH, Feedstock Composition, Nitrogen and Phosphorous added to Reactors 1 &amp; 2, Heat supplied to the reactors</td>
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</table>

Procedure (overall):

A 3-compartment water bath incubator and three, 1L model airtight anaerobic reactors, were constructed, using learning from previous science projects to regulate pH and temperature. The incubator (a modified, heated home aquarium) provided heating to maintain the reactors in a mesophilic temperature range. I used acetic acid or sodium bicarbonate to raise or lower the pH of the common starting slurry of kitchen and yard waste at the beginning of the reactor trials, and when any reactor exceeded 8.5 (limit of target bacteria growth). Nitrogen (urea) was added to Reactor 1, to achieve an approximate Carbon to Nitrogen mass ratio (C:N) of 25:1. Phosphorous (bonemeal) was added to Reactor 2, to achieve a Carbon to Phosphorous ratio (C:P) of 137.5:1. There was nothing added to Reactor 3, the control.

The nitrogen and phosphorus was as aforementioned provided as urea and bonemeal. The ratios of carbon to nitrogen, and carbon to phosphorus were, 25:1 for C:N and 137.5:1 for C:P. The urea and bonemeal, only 46.7% nitrogen, and 14% phosphorus were both recalculated to give 100% nitrogen to one, and 100% phosphorus to the other. (overall Urea:68.36g and Phosphorus:18.11g)
Two experimental runs (repeats) were conducted, lasting 25 and 18 days, to provide sufficient time for the methanogenic bacteria to reproduce and grow. A measure of the bacterial growth was done by viewing samples with a 1000 times magnification oil immersion microscope, and counting the bacteria in the field of view by using digital pictures of the samples. Sample counts were taken at the beginning middle and end of the experiment.

**Procedure (detailed):**

**Building:**

**Reactor Incubator:**
11) The reactor incubator was built using a 5-gallon aquarium wrapped in 1947.3 cm$^2$ of reflective insulation.
12) A cover for the reactor was made using 305cm2 of reflective insulation and duct tape, so that no heated air could escape.
13) Two 13x18cm pieces of wood were used to separate the reactors.
14) The incubator was heated with 4 100watt aquarium heaters, 1 in each compartment, and 1 extra in the middle compartment.
15) Six 2-inch slots were cut in the reflective insulation top cover so the tubes for slurry extraction, and gas release could get through.

**Reactor Design:**
1. Three 1L glass-canning jars and lids were used to make reactors.
2. Three holes were drilled in the top of each jar lid to so that 2 ¼ inch and 1 3/8 inch brass tube fittings could be secured in place, 1 for the thermometer, 1 for possible gas release, and the other for slurry extraction.
3. The hole for the slurry removal had an airlock valve system so no oxygen could ever get to the methanogenic bacteria, and the thermometer hole was secured with silicon.
4. Inside the slurry sample fitting had a 5.5-inch length of 3/8 of an inch plastic tubing.
5. To measure any gas output a water displacement system was in place, with the reactors, each attached to a 500mL flask and the flask attached to 250mL-graduated cylinders, with the exceptions of one being attached to a 100mL graduated cylinder, as another 250mL cylinder was not available.
6. The reactors in the reactor incubator were surrounded by water for convection heating.

**Experimental:**

**Slurry:**
- To provide slurry for the experiment 4.51L leaves/ grass, and 4.51L kitchen leavings were combined with 3L of water were used. The kitchen waste used was blended to maximize surface area for the aceto-bacters to convert it to acid.
- The slurry collected over a period of 2 weeks. The mix of the slurry was overall 3/8 kitchen waste, 3/8 grass and leaves, and 2/8 water.
- The slurry was then used to fill the 3 reactors with 596.412mL of slurry (calculations are In Appendix 1a)

**pH:**
1. To regulate pH readings were taken every three days, and at the beginning and end of each experiment.

2. If the pH was too high or low, an addition of 20mL of vinegar or sodium bicarbonate mixed with 30mL of water was added.

Nitrogen and Phosphorus:

9. To provide the ratios of 25:1 for C:N and 137.5:1 for C:P, the urea had to be calculated only with 46.7% of it (the nitrogen part) and 14% for bonemeal (the phosphoric acid part, which again had to be calculated for only phosphorus).

10. Overall, 68g of urea was added to one reactor and 18g of bonemeal to another, to achieve the required ratios. (calculations can be seen in Appendix 1b)

Materials:

- 1x CSA 120V Model # HV5-20 Oil immersion Microscope
- 1x 5 gallon aquarium
- 1x 100mL Graduated cylinder
- 3x 500mL glass flasks
- 3x meat thermometers
- 3x rubber stoppers
- 4x aquarium heaters (100 watts each)
- 2x 13 by 18cm wooden dividers
- 3x 1L canning jars
- 3x rubber edged canning lids
- 21 cm of ¼ inch plastic tubing
- 140 cm of 3/8 plastic tubing
- 1x 35cc syringe
- 2x 10cc syringes
- 20mL of vinegar
- 68g of urea
- 18g of bonemeal
- 1x pH meter
- 6x 3/8 inch brass tube fittings
- 6x ¼ inch brass tube fittings
- 2299.305 cm² of reflective insulation
- 3x ¼ inch brass valves
Results: Analysis was done of reactor temperature, pH, and methanogen production. Two trials were done, but the first trial did not have any methanogen counts at the beginning because no microscope was secured at that time. The calculations used to determine the C:N and C:P ratios are in Appendix 1b.

**Trial 1**

**Temperature:** In this experiment the temperature objective was to keep above 30°C (in the mesophilic temperature range) on average in all reactors. The average temperature for Reactor 1 (phosphorus) was 30.3°C, Reactor 2 (nitrogen) 32.6°C, Reactor 3 (control) 32.4°C. These temperatures were taken over 19 days out of 28 days of experimentation. The system was in control during the experiment, with no temperature drops below 30°C.

**pH:** The graph below shows the pH measurements of Trial 1. The pH measurements were not what were planned (the pH was to be between 6 – 8.5). The Reactor 1 average pH was 5.6 (it is fine for bacterial production, but not optimal), Reactor 2 was 7.7, which was right in the middle of the range, but the pH was at one point 8.9, and definitely bacteriostic (kills bacteria) (20mL of vinegar was added to bring it down to 8.2 which was within the desired range). Reactor 3’s average pH was slightly out of range at 5.9, but no control measures were taken.
Bacteria: In Trial 1 the beginning methanogen counts were not taken, as a microscope was not available at that time. Also all numbers are approximate as the numbers came from counting in photos and, possibly samples that did not happen to contain methanogens, or contained more than in true representation. The samples were taken at points in the system when there would most likely be bacteria growth. In Reactor 1 the first sample taken (on the 25th day) approximately 100 methanogens were counted, and at the end of Trial 1, approximately 136 methanogens were counted. Reactor 2’s first sample (that was also taken on the 25th day) contained approximately 58 methanogens, and at the end of Trial 1, approximately 59 were counted. Reactor 3’s sample taken at the same time as the previous ones, had approximately 50 methanogens, and at the end of Trial 1 had about 100 methanogens.
Trial 1 Bacteria Count Comparison

No Reactor 1 End picture (it was in movie format)

Ending Pictures

Trial 2

Temperature: In Trial 2 of the experiment the temperature was to follow the same temperature guidelines as mentioned above. All the reactors had an average temperature of 30°C plus, with none going below 30°C. Reactor 1’s (Nitrogen for this half) average temperature was 32°C. Reactor 2’s (Phosphorus for this half) average temperature was 32.7°C, and Reactor 3’s (still control) average temperature was 32.8°C. These numbers were taken over 15 of 18 days of Trial 2 experimentation (I was away on holiday for 3 days).
**pH:** The pH measurements for this half of the overall experiment went along as planned (6-8.5 pH range). Reactor 1’s average pH was 7.5, Reactor 2’s average pH was 6.1, and Reactor 3’s pH average was 6.4. The only measurement that went above the limits was the last one, though there is a gap where possibly it went higher.

**Bacteria:** In Trial 2 the methanogen counts were taken at the start (after two days of maturation), at approximately the middle and at the end. As also stated before all methanogen counts must looked at as estimates or indication as they were one-point samples. In Reactor 1, the first sample was approximately 32 methanogens; the second sample (taken on the 11th day) contained about 30 bacteria, and in the final sample contained approximately 56 methanogens. Reactor 2’s first sample contained approximately 36 methanogens, the second sample contained approximately 150 methanogens, and the final sample held 200 plus methanogens. In Reactor 3 the first sample yielded approximately 34 methanogens, the second sample held about 43 methanogens, and the final sample contained approximately 97-100 methanogens.

Andrew Darby 2
Trial 2 Bacteria Count Comparison

Sample Number

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Reactor 1 Nitrogen</th>
<th>Reactor 2 Phosphorus</th>
<th>Reactor 3 Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>43</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>200</td>
<td>97</td>
</tr>
</tbody>
</table>

Reactors 1, 2, and 3 show Ending Pictures (at 1000x in oil immersion lense)

Andrew Darby
Conclusions

The overall goal of this experiment was to see the effects of C:N and C:P ratios on methanogenic bacteria growth, as a preliminary work to see if it was feasible to study them with an optical microscope, and if the change in ratios would have an affect on methanogen populations. The hypothesis was only proved partially correct as Reactor 2 out produced Reactor 3 on both Trials, but Reactor 1 (the nitrogen induced one) only out produced Reactor 3, on the very first measurement of Trial 1. While I predicted that Reactor 1 would out produce Reactor 2, the opposite was observed taking place, and can be explained. Overall Reactor 2 was the highest producer overall with 200 plus bacteria at the end of Trial 2.

From the observations and data collected, it seems that phosphorus level achieved in the test was the most beneficial for methanogen growth (in both trials). Both Reactor 1 and Reactor 3 had steady increases of methanogen growth during the experiment. By increasing phosphorous above base levels, even in a small amount, appears to encourage bacteria growth relative to the control. This makes sense because methanogens use it for reproduction and cell growth; in the presence of sufficient carbon and nitrogen, it facilitates higher reproduction rates than the control.

The results of Reactor 1 were disappointing, but not completely unexpected because I did not have a way to quantify, but only estimate the initial nitrogen content. The 68g of urea added to provide the 25:1 ratio, combined with the nitrogen already present within the reactor environment was probably higher than the system could accommodate, and could be a source of error in the results when looked upon as a whole. However, it does point to the need for further research to better characterize the base slurry, and the ratios affecting it.

The need for temperature and pH control is to provide the best growing conditions for the methanogens so that the only thing affecting it is the C:N and C:P ratios. The temperature of the experiment was not a large variable, but simply an added factor to methanogen growth if kept within certain mesophilic parameters. The pH of reactors is one of the most important factors besides the nitrogen and phosphorus ratios. By controlling the pH to a neutral to slightly base mix, methanogens reproduce and thrive. By controlling these variables to provide the best growing conditions possible, it left all else to be determined by the C:N and C:P ratios.

Looking further at the nitrogen-enriched reactor, across both trials, the original hypothesis, that increasing available nitrogen relative to the base slurry was shown to be incorrect in the range I studied. The rise in pH as well as the observed ammonia smell at the end of the reaction, combined with what appeared to be good decomposition (visual) suggests that for the conditions in this experiment, the actual nitrogen content (mostly from green plant material) was near or at the optimal limit, and the added amount ultimately become limiting, as observed by the lower methanogen count. In Trial 2 Reactor 2 and 3, both produced possible biogas (there was condensation in the flasks and the water was somewhat lower, and water is a by-product of CH4 production). While these conclusions are disappointing, the project did fill the overall goal of seeing if this type of experiment was possible to do at home, using only optical methods of measuring bacteria counts. Also, since all Reactors in both Trials saw methanogen growth it suggests that an anaerobic environment was achieved and maintained. In the future, I hope to run experiments on different C:N ratios in relationship to their effects on methanogens, and also C:P ratios and their effect. These ideas
would also be coupled with the mixing of the ratios together to try and find almost perfect methanogen growing condition, which could possibly be utilized on a commercial scale. In conclusion, while the original hypothesis about the relative value of nitrogen versus phosphorous was not confirmed in this experiment, the research project, shows that it can be done on household scale, opens doors to other ratio research and its affect on the amazing bacteria/archaea called methanogens.

Error Analysis:

The largest source of potential error in this lab, was in the sampling representatives from the reactors (there was no way to sample at all levels of the reactor and so most of the samples came from the bottom of the jar. Also, a study of the error in counting methanogenic (as opposed to what seem to be aceto-bacters) in the field of view of the sample (and also reflecting the motility of the methanogens, which were moving in and out of the field of view), conducted at the beginning of the experiment suggest a counting absolute error of about +/- 5 bacteria. That is to say, in the results present, a difference of 10 or less is probably not directionally significant. Because of the limited runs in this experiment, I was not able yet to do a statistical comparison of runs under the same conditions. Repeats of runs would lower the relative error, and help establish confidence intervals.

Applications:

The knowledge and techniques developed in this experiment, are meant to lay foundations of a series of experiments into feedstock ratios in anaerobic decomposition, in the home. The testing of such feedstocks and ratios is important to the development of large quantity of biogas production digesters, which can metabolize waste faster, and produce higher quality biogas (more CH4). By experimenting on a laboratory scale, it is possible to apply these feedstock treatment must be done, to almost cultivate the slurry to provide near perfect conditions. Experiments testing the highest and lowest points in C:N and C:P, along with finding the best ratios together is needed to optimize methanogen and therefore biogas production. By doing this, it helps provide an economically friendly way to get rid of waste with practically non-existent harm to the environment.
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