Methane Production from Municipal Solid Waste

Introduction
Archaeological investigations of landfills have revealed that biodegradable wastes can be found — virtually intact — 25 years after burial. We know that landfills contain bacteria with the metabolic capability to degrade many of the materials that are common components of municipal refuse. The persistence for decades of degradable materials in the presence of such organisms appears somewhat paradoxical. In this experiment students will explore the factors that influence biodegradation of waste materials in landfills. Although recycling has significantly reduced the amount of landfill space dedicated to paper and other lignocellulosics, paper products are still a significant fraction of the solid waste stream. In this laboratory students will measure the rate and extent of anaerobic degradation of newsprint, Kraft paper, coated paper, and food scraps.

Theory
Over 150 million tons of municipal solid waste (MSW) are generated every year in the United States, and more than 70% of the MSW is deposited in landfills (Gurijala and Suflita 1993). Paper constitutes the major weight fraction of MSW, and this laboratory will focus on the biodegradation of that component. Anaerobic biodegradation of paper produces methane and carbon dioxide. Methane is a fuel and is the major component of natural gas. Methane produced in sanitary landfills represents a usable but underutilized source of energy. Energy recovery projects are frequently rejected because the onset of methane production is unpredictable and methane yields vary from 1-30% of potential yields based on refuse biodegradability data (Barlaz, Ham et al. 1992). The low methane yields are the result of several factors that conspire to inhibit anaerobic biodegradation including low moisture levels, resistance to biodegradation, conditions that favor bacterial degradation pathways that do not result in methane as an end product, and poor contact between bacteria and the organic matter.

Characteristics of municipal solid waste
The physical composition of residential municipal solid waste (MSW) in the United States is given in Table 6-1. The fractional

Table 6-1. Typical physical composition of residential MSW in 1990 excluding recycled materials and food wastes discharged with wastewater (Tchobanoglous, Theisen et al. 1993)

<table>
<thead>
<tr>
<th>Component</th>
<th>Range (%) by weight</th>
<th>Typical (%) by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>food wastes</td>
<td>6-18</td>
<td>9.0</td>
</tr>
<tr>
<td>paper</td>
<td>25-40</td>
<td>34.0</td>
</tr>
<tr>
<td>cardboard</td>
<td>3-10</td>
<td>6.0</td>
</tr>
<tr>
<td>plastics</td>
<td>4-10</td>
<td>7.0</td>
</tr>
<tr>
<td>textiles</td>
<td>0-4</td>
<td>2.0</td>
</tr>
<tr>
<td>rubber</td>
<td>0-2</td>
<td>0.5</td>
</tr>
<tr>
<td>leather</td>
<td>0-2</td>
<td>0.5</td>
</tr>
<tr>
<td>yard wastes</td>
<td>5-20</td>
<td>18.5</td>
</tr>
<tr>
<td>wood</td>
<td>1-4</td>
<td>2.0</td>
</tr>
<tr>
<td>Organic total</td>
<td>79.5</td>
<td></td>
</tr>
<tr>
<td>Inorganic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glass</td>
<td>4-12</td>
<td>8.0</td>
</tr>
<tr>
<td>tin cans</td>
<td>2-8</td>
<td>6.0</td>
</tr>
<tr>
<td>aluminum</td>
<td>0-1</td>
<td>0.5</td>
</tr>
<tr>
<td>other metal</td>
<td>1-4</td>
<td>3.0</td>
</tr>
<tr>
<td>dirt, ash, etc.</td>
<td>0-6</td>
<td>3.0</td>
</tr>
<tr>
<td>Inorganic total</td>
<td>20.5</td>
<td></td>
</tr>
</tbody>
</table>
contribution of the listed categories has evolved over time, with a trend toward a decrease in food wastes because of increased use of kitchen food waste grinders, an increase in plastics through the growth of their use for packaging, and an increase in yard wastes as burning has ceased to be allowed by most communities (Tchobanoglous, Theisen et al. 1993). Excluding plastic, rubber, and leather, the organic components listed in Table 6-1 are, given sufficient time, biodegradable.

Although recycling efforts divert a significant fraction of paper away from landfills, paper continues to be a major component of landfilled waste. The types of paper found in MSW are listed in Table 6-2.

The elemental composition of newsprint and office paper are listed in Table 6-3.

The major elements in paper are carbon, hydrogen, and oxygen that together constitute 93.5% of the total solids. The approximate molecular ratios for newspaper and office paper are $C_6H_9O_4$ and $C_6H_{9.5}O_{4.5}$ respectively.

**Biodegradation of cellulose, hemicellulose, and lignin**

Cellulose and hemicellulose are the principal biodegradable constituents of refuse accounting for 91% of the total methane potential. Cellulose forms the structural fiber of many plants. Mammals, including humans, lack the enzymes to degrade cellulose. However, bacteria that can break cellulose down into its subunits are widely distributed in natural systems, and ruminants, such as

<table>
<thead>
<tr>
<th>Type of paper</th>
<th>Range</th>
<th>Typical</th>
</tr>
</thead>
<tbody>
<tr>
<td>newspaper</td>
<td>10-20</td>
<td>17.7</td>
</tr>
<tr>
<td>books and magazines</td>
<td>5-10</td>
<td>8.7</td>
</tr>
<tr>
<td>commercial printing office paper</td>
<td>4-8</td>
<td>6.4</td>
</tr>
<tr>
<td>other paperboard</td>
<td>8-12</td>
<td>10.1</td>
</tr>
<tr>
<td>paper packaging</td>
<td>6-10</td>
<td>7.8</td>
</tr>
<tr>
<td>other nonpackaging paper</td>
<td>8-12</td>
<td>10.6</td>
</tr>
<tr>
<td>tissue paper and towels</td>
<td>4-8</td>
<td>5.9</td>
</tr>
<tr>
<td>corrugated materials</td>
<td>20-25</td>
<td>22.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Newsprint</th>
<th>Office Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>49.1%</td>
<td>43.4%</td>
</tr>
<tr>
<td>H</td>
<td>6.1%</td>
<td>5.8%</td>
</tr>
<tr>
<td>O</td>
<td>43.0%</td>
<td>44.3%</td>
</tr>
<tr>
<td>NH$_4$-N</td>
<td>4 ppm</td>
<td>61 ppm</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>4 ppm</td>
<td>218 ppm</td>
</tr>
<tr>
<td>P</td>
<td>44 ppm</td>
<td>295 ppm</td>
</tr>
<tr>
<td>PO$_4$-P</td>
<td>20 ppm</td>
<td>164 ppm</td>
</tr>
<tr>
<td>K</td>
<td>0.35%</td>
<td>0.29%</td>
</tr>
<tr>
<td>SO$_4$-S</td>
<td>159 ppm</td>
<td>324 ppm</td>
</tr>
<tr>
<td>Ca</td>
<td>0.01%</td>
<td>0.10%</td>
</tr>
<tr>
<td>Mg</td>
<td>0.02%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Na</td>
<td>0.74%</td>
<td>1.05%</td>
</tr>
<tr>
<td>B</td>
<td>14 ppm</td>
<td>28 ppm</td>
</tr>
<tr>
<td>Zn</td>
<td>22 ppm</td>
<td>177 ppm</td>
</tr>
<tr>
<td>Mn</td>
<td>49 ppm</td>
<td>15 ppm</td>
</tr>
<tr>
<td>Fe</td>
<td>57 ppm</td>
<td>396 ppm</td>
</tr>
<tr>
<td>Cu</td>
<td>12 ppm</td>
<td>14 ppm</td>
</tr>
</tbody>
</table>
cows, have these microorganisms in their digestive tract. Cellulose is a polysaccharide that is composed of glucose subunits (see Figure 6-1).

Another component of the walls of plants is hemicellulose, which sounds similar to cellulose but is unrelated other that that it is another type of polysaccharide. Hemicelluloses made up of five carbon sugars (primarily xylene) are the most abundant in nature.

Lignin is an important structural component in plant materials and constitutes roughly 30% of wood. Significant components of lignin include coniferyl alcohol and syringyl alcohol subunits (Figure 6-2).

The exact chemical structure of lignin is not known but its reactivity, breakdown products, and the results of spectroscopic studies reveal it to be a polymeric material containing aromatic rings with methoxy groups (-OCH₃) (Tchobanoglous, Theisen et al. 1993). One of the many proposed structures for lignin is shown in Figure 6-3.

Degradation of lignin requires the presence of moisture and oxygen and is carried out by filamentous fungi (Prescot, Harley et al. 1993). The biodegradability of lignocellulosic materials can be increased by an array of physical/chemical processes including pretreatment to increase surface area (size reduction), heat treatment, and treatment with acids or bases. Such treatments are useful when wood and plant materials are to be anaerobically degraded to produce methane. Research on this topic has been performed by Cornell Prof. James Gossett (Gossett and McCarty 1976; Chandler, Jewell et al. 1980; Gossett, Stuckey et al. 1982;
Three major groups of bacteria are involved in the conversion of cellulosic material to methane (Zehnder 1978): (1) the hydrolytic and fermentative bacteria that break down biological polymers such as cellulose and hemicellulose to sugars that are then fermented to carboxylic acids, alcohols, carbon dioxide and hydrogen gas, (2) the obligate hydrogen reducing acetogenic bacteria that convert carboxylic acids and alcohols to acetate and hydrogen, and (3) the methanogenic bacteria that convert primarily acetate and hydrogen plus carbon dioxide to methane. Sulfate reducing bacteria (SRB) may also play a role in the anaerobic mineralization of cellulosic material. In the presence of sulfate, the degradation process may be directed towards sulfate reduction by SRB with the production of hydrogen sulfide and carbon dioxide (Barlaz, Ham et al. 1992).

**Cellular requirements for growth**

The availability of oxygen is a prime determinant in the type of microbial metabolism that will occur. Microbial respiration of organic carbon is a combustion process, in which the carbon is oxidized (i.e., is the electron donor) in tandem with the reduction of an electron acceptor. The energy available to microorganisms is greatest when oxygen is used as the electron acceptor and therefore aerobic metabolic processes will dominate when oxygen is available. Some microorganisms require oxygen to obtain their energy and are termed "obligate aerobes." In the absence of oxygen, other electron acceptors such as nitrate (NO₃⁻), sulfate (SO₄²⁻) and carbon dioxide (CO₂) can be used. Organisms that can only exist in an environment that contains no oxygen are termed "obligate anaerobes." Organisms that have the ability to grow in both the presence and the absence of oxygen are said to be "facultative."

The availability of nutrients can limit the ability of cells to grow and consequently the extent of biodegradation. Nitrogen and/or phosphorous constitute important nutrients required for cell synthesis. Inorganic bacterial nutritional requirements also include sulfur, potassium, magnesium, calcium, iron, sodium and chloride. In addition, inorganic nutrients needed in small amounts (minor or trace nutrients) include zinc, manganese, molybdenum, selenium, cobalt, copper, nickel, vanadium and tungsten. Organic nutrients (termed "growth factors") are also sometimes needed (depending on the microorganism) and include certain amino acids, and vitamins (Metcalf & Eddy 1991).

Environmental conditions such as pH, temperature, moisture content, and salt concentration can have a great influence on the ability of bacteria to grow and survive. Most bacteria grow in the pH range from 4.0 to 9.5 (although some organisms can tolerate more extreme pH values), and typically grow best in the relatively narrow range from 6.5 to 7.5 (Metcalf & Eddy, 1991). Microorganisms have a temperature range over which they function best, and are loosely characterized as psychrophilic (ability to grow at 0°C), mesophilic (optimal growth at 25-40°C) or thermophilic (optimal growth above 45-50°C) (Brock 1970). Many common methanogens are mesophilic. Elevated temperatures also favor faster reaction rates.

While some microorganisms are very tolerant of low moisture conditions, active microbial growth and degradation of organic matter necessitates that water not be a scarce resource. Cells take water in through their semi-permeable membrane surface
by osmosis. This uptake mechanism requires that the solute concentration inside the cell be higher than that of the outside media. Organisms that grow in dilute solutions can not tolerate high salt concentrations because their normal osmotic gradient is reversed and they can not take in water. Some cell strains, termed “halophiles” are adapted for growth at very high salt concentrations.

The above factors suggest that bacterial degradation of MSW to produce methane will occur optimally at circumneutral pH, low ionic strength, in the absence of oxygen, nitrate and sulfate, in the presence of moisture and nutrients, and under mesophilic conditions.

**Estimates of paper biodegradability**

Volatile solids (VS) content (determined by weight loss on ignition at 550°C) has been used to estimate the biodegradability of MSW components, but this measure overestimates the biodegradability of paper. Paper products have a very high volatile solids content. Newsprint, office paper, and cardboard have VS of 94%, 96.4%, and 94% respectively (Tchobanoglous, Theisen et al. 1993). Paper products also can have a high content of lignocellulosic components that are only slowly degradable. Lignin constitutes approximately 21.9%, 0.4% and 12.9% respectively of the VS in newsprint, office paper, and cardboard. Lignin content and biodegradability are strongly correlated and thus lignin content can be used to estimate biodegradability and potential methane production. Chandler et al. (1980) found a relationship between lignin content and biodegradable volatile solids using a wide variety of waste materials. The empirical relationship suggests that not only is lignin not easily biodegraded, but that lignin also reduces the biodegradability of the nonlignin components. This reduction in biodegradability may be caused by lignin polymeric material physically preventing enzymatic access to the nonlignin components. The relationship is

\[
\text{VS}_{\text{biodegradable}} = -2.8\text{VS}_{\text{lignin}} + 0.83
\]

where \(\text{VS}_{\text{biodegradable}}\) is the biodegradable fraction of the volatile solids and \(\text{VS}_{\text{lignin}}\) is the fraction of volatile solids that are lignin. From equation 6.1 the maximum destruction of VS is limited to about 83%, a limitation due to the production of bacterial by-products. The high concentration of lignin in newsprint makes it much less biodegradable than more highly processed office paper (Table 6-4).

**Energy recovery from MSW**

Energy could be recovered from MSW by direct combustion in an incinerator or by anaerobic biodegradation and production of methane. Proximate analysis is used to measure moisture content, volatile matter, fixed carbon (combustible but not volatile), and ash. Proximate analysis can be used to predict ash

![Table 6-4. Biodegradability of selected components of MSW (Tchobanoglous, Theisen et al. 1993)](image)

<table>
<thead>
<tr>
<th>Type of waste</th>
<th>VS/TS</th>
<th>Lignin/VS</th>
<th>VS\text{biodegradable}*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixed food</td>
<td>7-15</td>
<td>0.4</td>
<td>82</td>
</tr>
<tr>
<td>newsprint</td>
<td>94</td>
<td>21.9</td>
<td>22</td>
</tr>
<tr>
<td>office paper</td>
<td>96.4</td>
<td>0.4</td>
<td>82</td>
</tr>
<tr>
<td>cardboard</td>
<td>94.0</td>
<td>12.9</td>
<td>47</td>
</tr>
</tbody>
</table>

* Obtained by using equation 6.1
production from incineration. The energy content is measured in a bomb calorimeter. Proximate analysis results and energy content of MSW are given in Table 6-5.

Gas production from anaerobic digestion is typically 30% CO₂ and 70% CH₄. The methane is a valuable fuel and has an energy content of 802.3 kJ/mol or 50 MJ/kg. The combustion of methane produces only carbon dioxide and water.

Because paper products are a major fraction of MSW and paper energy content is significant, the majority of energy in MSW is contained in paper products. Incineration or methane production can be used to capture some of this available energy.

$$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$$  

6.2

Effect of MSW particle size

The large size of pieces of MSW is suspected to decrease the ability of microbes to degrade the material. Landfill gas production has been correlated with refuse particle size (Ferguson 1993). The effect of particle size reduction was initially explained by the resultant increase in surface area available for microbial attach. Laboratory studies under saturated conditions, however, suggest that size reduction, even down to a few microns or tens of microns has little effect on the rate of degradation. According to Ferguson (1993), surface area increases only slightly with decreasing particle size for platey and fibrous particles such as paper. Thus the effect of size reduction on the methane production in landfills may be that relatively large pieces of plastic, paper, or other material shield the materials beneath them from infiltrating water. The shielded material may remain too dry for biodegradation. Pulverization breaks down the impermeable barriers and more of the waste is exposed to water (Ferguson 1993).

Potential methane production from municipal solid waste

Under anaerobic conditions microorganisms can produce both CO₂ and CH₄ (methane) without consuming any oxygen. Other significant end products include odorous gases such as ammonia (NH₃), and hydrogen sulfide (H₂S) (see Figure 6-4). Because anaerobic biodegradation produces gas it is possible to monitor the extent and rate of anaerobic biodegradation by measuring gas production (Suflita and Concannon 1995).
Gas production

Because anaerobes get relatively little energy from the organic matter their conversion of carbon to cell material (synthesis) is much lower than for aerobes. Typically 10% of the organic matter may be converted to anaerobe cell mass. Thus the majority of the biodegraded organic matter is converted to gas and the gas production can be used as a measure of biodegradation. The ideal gas law is used to determine the moles of gas produced from the pressure, volume, and temperature.

\[ n = \frac{PV}{RT} \]  
\[ \Delta n = \frac{\Delta PV}{RT} \]

where \( \Delta P \) is the change in pressure relative to the initial pressure in the bottle.

In these experiments the bottle volume is 120 mL and the maximum recommended pressure increase is 80 kPa (12 psi). The volume of liquid in the bottles is 20 mL and the volume contributed by solids is expected to be negligible. Thus the nominal volume of gas in the bottles will be 100 mL. Solving for the number of moles of gas (CH\textsubscript{4} and CO\textsubscript{2}) produced by anaerobic digestion

\[ \Delta n = \left( \frac{80 \times 10^5 Pa}{8.31 \frac{Pa \cdot m^3}{mol \cdot K}} \right) \left( \frac{100 \times 10^{-6} m^3}{308 K} \right) = 3.13 \text{ mmole C} \]

The molecular formula of cellulose is C\textsubscript{6}H\textsubscript{10}O\textsubscript{5} and thus 27 g of cellulose has 1 mole of carbon. The relation obtained in equation 6.5 is used to determine the maximum amount of cellulose that can be anaerobicly degraded without exceeding 80 kPa in the bottles.

\[ 3.13 \text{ mmole C} \cdot \frac{27 \text{ mg cellulose}}{\text{mmole C}} = 84 \text{ mg cellulose} \]

The mass of paper containing 84 mg of biodegradable cellulose can be obtained using Table 6-4 and the results of equation 6.6. The mass of dry newspaper that will produce a pressure increase of 80 kPa is
Similar calculations can be performed for other types of waste. The maximum mass of glucose (CH₂O has 30 g of glucose per mole of carbon) is

\[
\frac{3.13 \text{ mmole C}}{\text{mmole C}} \times \frac{30 \text{ mg glucose}}{3.13 \text{ mmole C}} = 94 \text{ mg glucose}
\]

Although glucose is expected to be completely biodegradable, a small amount of glucose will be converted into refractory cell byproducts.

The above calculations are based on the assumption that all of the gas produced is volatile and is not dissolved. Carbon dioxide is soluble and thus some of the CO₂ produced will be dissolved and will not result in increased pressure.

**Acid neutralizing capacity requirements**

The high partial pressure of CO₂ resulting from anaerobic biodegradation causes a high concentration of carbonic acid \( [H₂CO₃^+] \) and thus would result in a reduced pH if there were insufficient Acid Neutralizing Capacity (ANC). The amount of ANC required to counteract the high partial pressure of CO₂ can be obtained from the Henry’s constant for dissolution of CO₂, and from the dissociation constant for carbonic acid.

\[
K_H = \frac{[H₂CO₃^+]}{p_{CO₂}}
\]

where \( K_H \) has a value of \( 3.12 \times 10^{-4} \) moles/J. The first dissociation constant for carbonic acid is

\[
K_1 = \frac{[H⁺][HCO₃^-]}{[H₂CO₃^+]}
\]

where \( K_1 \) has a value of \( 10^{-6.3} \). The definition of ANC for a carbonate system in equilibrium with the gas phase is

\[
ANC = \frac{p_{CO₂}K_H}{\alpha_0} \left( \alpha_1 + 2\alpha_2 \right) + \frac{K_w}{[H⁺]} - [H⁺]
\]

Where \( \alpha_0, \alpha_1, \alpha_2 \) are the fractions of total carbonate present as carbonic acid \( [H₂CO₃^+] \), bicarbonate \( [HCO₃^-] \), and carbonate \( [CO₃^{2-}] \) respectively and \( K_w \) is the dissociation constant for water. At circumneutral pH the hydrogen ion, hydroxide ion, and carbonate ion concentrations are negligible and equation 6.11 simplifies to

\[
ANC = \frac{p_{CO₂}K_H\alpha_1}{\alpha_0}
\]
The ratio of bicarbonate to carbonic acid may be determined from equation 6.10. Solving for the ratio of bicarbonate to carbonic acid:

\[
\frac{[HCO_3^-]}{[H_2CO_3]} = \frac{\alpha_1}{\alpha_0} = \frac{K_1}{[H^+]}
\]

Equation 6.13 can be substituted into equation 6.12 to obtain

\[
ANC = \frac{P_{CO_2} K_n K_1}{[H^+]}
\]

An estimate of the ANC required to maintain a neutral pH under a pressure of 30 kPa of CO2 can be obtained by substituting appropriate values into equation 6.14.

\[
ANC \equiv \left(3 \times 10^4 \text{ Pa}\right) \left(3.12 \times 10^{-4} \text{ moles} \frac{\text{N} \cdot \text{m}}{10^{-7} \text{ M}}\right)\]

\[
ANC \equiv 47 \text{ meq} / \text{L}
\]

The basal medium that will be used in this laboratory contains 71 meq/L ANC from sodium bicarbonate. If the pressure of CO2 reaches 60 kPa (30 kPa initial pressure plus 30 kPa from the production of CO2 during an experiment) then solving equation 6.14 for pH shows that (given the 71 meq ANC in the basal medium and a CO2 pressure of 60 kPa) the pH will drop to 6.88. Thus, the basal medium is sufficiently buffered to protect against significant pH changes.

**Carbon dioxide solubility**

At pH less than \(\approx 9\) the inorganic carbon will partition into three species, gaseous \(CO_2\), aqueous \(H_2CO_3^+\), and aqueous \(HCO_3^-\).

\[
n_{CO_2(g)} = n_{CO_2(l)} + n_{H_2CO_3^+} + n_{HCO_3^-}
\]

The number of moles of the inorganic carbon species can be determined based on the partial pressure of \(CO_2\), the ANC of the liquid and the gas and liquid volumes. The moles of gaseous \(CO_2\) is obtained from the ideal gas law

\[
n_{CO_2(g)} = \frac{P_{CO_2} V_g}{RT}
\]

The number of moles of \(H_2CO_3^+\) is obtained from the Henry’s law constant.

\[
n_{H_2CO_3^+} = P_{CO_2} K_n V_l
\]

The concentration of bicarbonate, \(HCO_3^-\), is equal to the ANC (for pH < 9).

\[
n_{HCO_3^-} = ANC \cdot V_l
\]
The total number of moles of inorganic carbon is the sum of the three species.

\[ n_{CO_2_{(total)}} = \frac{P_{CO_2} V}{RT} + \left( P_{CO_2} K_H + ANC \right) V \]

6.20

Therefore, the number of moles of inorganic carbon in an enclosed volume is a linear function of the partial pressure of \( CO_2 \) (Figure 6-5).

The pH will change as the partial pressure of \( CO_2 \) changes as shown in equation 6.14. Solving for the concentration of hydrogen ions equation 6.14 becomes

\[ [H^+] = \frac{P_{CO_2} K_H K_i}{ANC} \]

6.21

The relationship between pH and partial pressure of \( CO_2 \) is shown in Figure 6-5.

The basal medium to be used in this experiment will be purged with a 30:70 mixture of carbon dioxide and nitrogen prior to use. As shown in Figure 6-5 the pH of the basal medium is expected to rise to approximately 7.17. The headspace will also be purged with the same gas mixture and thus there will be 5.6 mmol of inorganic carbon in the bottles initially. After the anaerobic biodegradation has gone to completion, the carbon dioxide concentration will be measured by gas chromatography and the gas pressure by pressure sensors and thus the partial pressure of carbon dioxide will be known. Figure 6-5 or equation 6.20 can be used to determine the final mass of inorganic carbon in the bottles. The difference between the initial and final inorganic carbon concentration can be used to determine the amount of organic carbon converted to carbon dioxide.

**Methane solubility**

The Henry’s constant for methane (\( K_{H(CH_4)} \)) at 25°C is 1.48 x 10^{-5} mol/J (Mackay and Shiu 1981). Methane is significantly less soluble than carbon dioxide and does not form other soluble aqueous species. The mass of gaseous and dissolved methane is given by equations 6.22 and 6.23.
\[ n_{CH_4(g)} = \frac{P_{CH_4}V_g}{RT} \] 6.22

\[ n_{CH_4(aq)} = \frac{P_{CH_4}K_{H(CH_4)}V_l}{V_i} \] 6.23

The ratio of the mass of gaseous to dissolved methane gives an indication of the significance of dissolved methane.

\[ \frac{n_{CH_4(g)}}{n_{CH_4(aq)}} = \frac{V_g}{V_iK_{H(CH_4)}RT} \] 6.24

Substituting appropriate values into equation 6.24

\[ \frac{n_{CH_4(g)}}{n_{CH_4(aq)}} = \frac{V_g}{V_i \left(1.48 \times 10^{-5} \text{ mol J} \right) \left(8.31 \text{ J mol}^{-1} \text{ K}^{-1} \right) \left(308 \text{ K} \right)} \] 6.25

If the gas and liquid volumes are approximately equal there will be approximately 26 times as much methane in the gaseous phase as in the dissolved phase. This ratio is independent of the methane partial pressure. The total number of moles of methane can be obtained from the partial pressure of methane in the gaseous phase.

\[ n_{CH_4(total)} = P_{CH_4} \left(\frac{V_g}{RT} + K_{H(CH_4)}V_l \right) \] 6.26

The partial pressure of methane will be determined from the pressure in the bottle and mass of methane as measured by the gas chromatograph.

**Temperature effects**

The temperature of the bottles directly affects the pressure of gas as well as influences the rate of gas production by the microbes. Cummings and Stewart found that methane production was sharply inhibited by temperatures in excess of the optimum (37°C) and was undetectable at 20°C (1995). However, Suflita and Concannon (1995) reported anaerobic digestion at “room temperature” over a period of 2 months. If desired a constant temperature water bath can be used to keep all of the digesters at a constant and optimal temperature (35°C) for anaerobic degradation.

**Experiment description**

The experimental setup is a flexible system for obtaining data on the anaerobic decomposition of various organic materials by measuring the pressure of the gas produced. A schematic of the experimental setup is shown in Figure 6-6.
Bacterial degradation of selected materials will be assayed by placing known quantities in 120 mL bottles, inoculating with an active anaerobic mixed culture, sealing the bottles with rubber septa and aluminum crimp caps, and monitoring gas pressure and composition over time. Anaerobic digester supernatant from the Ithaca Wastewater Treatment Plant will be used as a source of microbes. The bottles will be monitored for biogas production with pressure sensors connected with a needle through the septa (see Figure 6-6). Gas composition will be determined by periodic analysis of methane (CH₄) and carbon dioxide (CO₂) via gas chromatography with a thermal-conductivity detector.

Gas production measurements will be automated by using pressure sensors in a procedure comparable to that described by Suflita and Concannon (1995). With this technique, a large number of bottles can be monitored with automated data acquisition by a single computer, allowing a wide variety of chemical and environmental parameters to be explored. The automated acquisition of gas data is necessary due to the numbers of bottles and length of incubation (ca. 4 weeks) anticipated. This experiment will be set-up and left virtually unattended while other laboratory exercises continue in intervening weeks.

Each sample type should be cut into small enough pieces to easily insert into the bottle. Students may also be interested in exploring biodegradation of other organic components of municipal solid waste (banana peels, rags, plastic bags, etc.). Table 6-6 suggests one configuration of several sample types. Each sample will receive 15 mL of basal medium.

One control should be “unamended” (i.e., with no waste) and contain the microbial inoculum and the O₂-free water to monitor any gas production attributable just to the added sludge, one “positive”

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sample Size</th>
<th>Inoculum</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>environmental control</td>
<td>none</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>bacterial control</td>
<td>none</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>positive control (glucose)</td>
<td>90</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>filter paper</td>
<td>50</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>cardboard</td>
<td>?</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>office paper</td>
<td>?</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>newsprint</td>
<td>?</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Student selected organic</td>
<td>?</td>
<td>5</td>
<td>up to 4</td>
</tr>
</tbody>
</table>

Table 6-6. Suggested sample preparation.
Each group does 2 sample types with 2 replicates. Each section does 2 water and 2 inoculum controls.
control should be the microbial inoculum, plus 90 milligrams of glucose and the 
O$_2$-free water (to verify that the microbial population is active in the added sludge),
and one control should be plain O$_2$-free water to control for variations in temperature,
and air pressure. The sample sizes of the various samples should be determined so 
that the bottles will not generate pressure greater than 80 kPa.

**Experimental methods**

**Safety concerns**

1) Municipal wastewater sludge will be used as a source of microbes. The sludge 
may contain biological and/or chemical hazards and should be handled 
accordingly.

2) Biological production of gas will generate pressure in a closed container. Testing 
has shown that this system is safe up to at least 200 kPa (30 psi). At 
approximately this pressure the needle is typically forced out of the septa. If the 
bottle is not vented the pressure can increase until the crimp cap is forced off. 
Bottles should not be capped for very long before the needles are inserted and 
pressure monitoring begins. The pressure trends should be monitored and, if 
excessive pressures are produced, the bottles must be vented and/or the 
temperature of the bath may be reduced.

3) Sharp needles are used in the experimental setup and precautions should be taken 
to avoid puncturing unintended objects (including students).

**Analysis of moisture content and volatile solids**

The fraction of volatile solids in the paper samples is the maximum that could 
possibly be degraded. Note that paper products cannot be ashed accurately because 
the strong flames easily carry some of the ashes away. Steps for sample moisture 
content and volatile solids fraction follow:

1) Weigh an aluminum boat

2) Weigh the aluminum boat with an organic sample (boat + water + VS + ash)

3) Dry in the 105°C oven

4) Cool in a desiccator

5) Weigh (boat + VS + ash)

6) Ash in the 550°C muffle furnace

7) Cool in a desiccator

8) Weigh (boat + ash)

**Sample preparation**

The bottles will be purged initially with a mixture of 30% CO$_2$ and 70% N$_2$ to 
remove any O$_2$ and to establish an initial carbon dioxide concentration so that the 
initial pH is not excessively high. If no carbon dioxide were present in the purging
gases the carbonic acid would be stripped out of solution and the pH would rise in the
basal medium.

**Acquisition of pressure data**

The biogas pressure will be measured indirectly by the pressure sensors. The
specified sensors work from zero to 100 kPa (6.89476 kPa/psi). They will withstand
2.5 times rated pressure (i.e., 250 kPa) but their output may be erroneous above the
upper limit of the working range. The output of the pressure sensors is zero to 0.100
volts with 0.100 volts indicating approximately 100 kPa. The outputs of the sensors
are fed through a 32-channel multiplexer/signal conditioner, an A/D converter board
and are monitored using LabVIEW software.

**Gas chromatograph analysis for separation of CO₂, CH₄ and N₂ (Optional)**

Permanent gases can be analyzed using a thermal conductivity detector (TCD) on a
gas chromatograph. The thermal conductivity detector measures the rate at which heat
is transported from the detector. If a gas with a different thermal conductivity than the
carrier gas passes through the detector a peak is detected. A flame ionization detector
could be used to measure methane, but would not be able to detect carbon dioxide or
nitrogen since they do not burn. A micropacked column containing packing designed
for analyses of permanent gases and light hydrocarbons (Supelco Carboxen 1004) is
used to separate the gases.

The TCD must be calibrated with known masses of the gases of interest. Nitrogen,
carbon dioxide and methane are available as compressed gases and can be sampled at
atmospheric pressure by opening a valve in a compressed gas line slightly and
sampling the discharge with a gas tight syringe. The ideal gas law is used to calculate
the moles of gas. The current atmospheric pressure in Ithaca is available through the
pressure is reported in inches of mercury it can be converted to Pascals by
multiplying by 3386 Pascals/Inch of Hg. The temperature of the laboratory is
available from the pH meters equipped with temperature probes. If a 100 µL gas
sample is used, the atmospheric pressure is 100 kPa, and the temperature is 22°C then
the number of moles of gas are calculated as:

\[
n = \frac{(100,000 \text{ Pa}) \left(100 \times 10^{-9} \text{ m}^3\right)}{8.31 \text{Pa} \cdot \text{m}^3 \cdot \text{mol} \cdot \text{K}^{-1} \cdot (295 \text{K})} = 4.08 \mu\text{mol}
\]

The number of moles of gas is independent of the type of gas. The relationship
between peak area and moles of gas is calculated by analyzing a known number of
moles of each gas. The TCD response will be different for each gas since the thermal
conductivity of each gas is different.

**Experimental method (short version)**

1) Dry 2 – 2 g samples for each sample type in the 105°C oven.
2) Take dried organic sample from the oven.
3) Keep 1 dried sample and determine the VS of the other sample.
4) Weigh appropriate amounts of the various dried samples for methane production.
5) Load bottles with organic samples (cut to smaller size as needed).
6) Add 15 mL of basal medium to each of the bottles.
7) Add 5 mL of inoculum to each of the bottles.
8) Purge the headspace of the bottles with an oxygen-free gas stream that is 30% CO₂ and 70% N₂.
9) Seal the bottles.
10) Insert the pressure sensor hypodermic needle into the bottle.
11) Sample the bottle pressures using the data acquisition software (take samples every hour and save the data as \Enviro\enviro\Courses\453\methane\pressure).

Gas Analysis Method
1) Calibrate the gas chromatograph using methane and carbon dioxide and using 20 μL samples
2) Take an initial headspace gas sample and analyze it using the gas chromatograph.
3) Sample gas composition after gas production has ceased using the gas chromatograph.

Prelab questions
1) Estimate the mass of cardboard and the mass of office paper that will produce a pressure rise of 80 kPa in the sample bottles at 35°C if the headspace volume is 100 mL. Use the predicted biodegradability based on the lignin content of the paper.

Data analysis
Perform the analysis on the data from the entire class.
1) Correct the inoculum controls for fluctuations in atmospheric pressure. How much gas was produced by the inoculum control? Note that the initial pressures have small variations between samples that may have been caused by the temperature of the bottle when it was sealed.
2) Correct all of the sample pressure data for fluctuations in atmospheric pressure and inoculum gas production by subtracting the inoculum controls. Note that you have several options here. Justify your method for handling the inoculum correction.
3) Compare the replicate samples by comparing the final pressure divided by the volatile solids that were added to the bottles initially. Are the replicates similar? Were there any cases where the replicates weren’t similar? What might have caused any differences? Is there any data that you should discard in further analysis? For any data that you discard explain why it should be discarded.
4) Develop a technique to estimate the time it took for the methane production to reach completion. Do you see any correlations between the type of sample and the time it took for the reaction to reach completion? If you don’t see any correlations, what factors may have been important in controlling the reaction rate?

5) Compare volatile solids and gas production by converting the mass of volatile solids to moles of carbon. You may assume that glucose and food is 40% carbon by mass and that samples with cellulose are 45% carbon by mass. Convert gas pressure into moles of carbon. Calculate and plot the fraction of volatile solids degraded as a function of time for each sample.

6) Compare (bar plot?) the fuel value of the methane produced with the fuel value of the original sample for each of the samples. Use the estimates of the original fuel value from Table 6-5 and the measured methane production. The fuel value of glucose is 424.7 KJ/mole C. Assume 70% of the gas produced was methane.

Optional Analysis Requiring Gas Composition

1) Calculate the moles of CO₂ and CH₄ produced by your samples based on the gas chromatograph analysis. Include the effect of carbon dioxide solubility. Use the basal medium control to subtract the initial headspace as well as any gas production by the inoculum.

2) Calculate the final pressure based on the GC measurements and compare with the pressure transducer measurements. Remember that the pressure transducer measured gage pressure.

References


**Lab Prep Notes**

### Setup

1) Use anaerobic digester supernatant as inoculum source. Place supernatant under fume hood. Use 5 mL per sample.

2) Setup 10 port purger with CO\textsubscript{2} and N\textsubscript{2} gas metered through rotometers. The top ball should be at 24 mm for CO\textsubscript{2} and at 84 mm for N\textsubscript{2}.

3) Set the GC with 300 Kpa column pressure, 180\textdegree{}C oven, 250\textdegree{}C injector and detectors, and 1.2 minute run time. Use 20 µL sample. The gases should come out in the order N\textsubscript{2}, CH\textsubscript{4}, and CO\textsubscript{2} at 0.44, 0.72, and 1 minute respectively. (Only if you are doing the optional GC analysis)

4) 4 samples/group plus 2 inoculum blanks and 2 water blanks.

### Class Plan

1) Sign up for samples

2) Each group chooses 2 types of samples

3) Dry samples in oven

4) Ash 1 of the 2 samples

### Equipment list

<table>
<thead>
<tr>
<th>Description</th>
<th>Vender</th>
<th>Catalog</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 µl syringe w/valve</td>
<td>Supelco</td>
<td>2-2272</td>
</tr>
<tr>
<td>side port needle</td>
<td>Supelco</td>
<td>2-2289</td>
</tr>
<tr>
<td>Carboxen 1004 micropacked column</td>
<td>Supelco</td>
<td>1-2846</td>
</tr>
<tr>
<td>Hp 5890 Series II GC TCD kit</td>
<td>Hewlett-Packard</td>
<td>5890A</td>
</tr>
<tr>
<td>1/8&quot; column adapter pressure regulators</td>
<td>Hewlett-Packard</td>
<td>19232E option 095</td>
</tr>
<tr>
<td>RS232C board Helium</td>
<td>Hewlett-Packard</td>
<td>L43 option 560</td>
</tr>
<tr>
<td>Wrist action Shaker Vials</td>
<td>Fisher Scientific</td>
<td>14-260</td>
</tr>
<tr>
<td>Aluminum crimp tops Butyl stopper</td>
<td>Fisher</td>
<td>03-375-23C</td>
</tr>
<tr>
<td>Crimping tool EPDM stoppers-13x20 mm luer lock needles</td>
<td>Fisher</td>
<td>03-375-22AA</td>
</tr>
<tr>
<td>21 gauge Pressure transducer, 0 to 15 psig</td>
<td>Fisher</td>
<td>3-3280</td>
</tr>
<tr>
<td>12 V DC Power supply</td>
<td>Omega</td>
<td>PX136-015GV</td>
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<tr>
<td>Incubator</td>
<td>Omega</td>
<td>PSS-12</td>
</tr>
<tr>
<td>Multiplexer</td>
<td>National Instruments</td>
<td>11-690-650D</td>
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<tr>
<td>4 slot chassis SCXI-1000</td>
<td>National Instruments</td>
<td>776570-01</td>
</tr>
<tr>
<td>32 channel SCXI-1100</td>
<td>National Instruments</td>
<td>776572-00</td>
</tr>
<tr>
<td>SCXI-1200 parallel port</td>
<td>National Instruments</td>
<td>776783-00</td>
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</table>
Table 6-11. Gas chromatograph conditions

<table>
<thead>
<tr>
<th>gas</th>
<th>pressure</th>
<th>flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>carrier (He)</td>
<td>kPa</td>
<td>5 mL/min</td>
</tr>
<tr>
<td>Ref</td>
<td></td>
<td>15 mL/min</td>
</tr>
<tr>
<td>temperatures</td>
<td>°C</td>
<td></td>
</tr>
<tr>
<td>oven (isothermal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injector</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>TCD</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Column</td>
<td>Supplier</td>
<td>Catalog number</td>
</tr>
<tr>
<td>Carboxen 1004 micropacked column</td>
<td>Supelco</td>
<td>1-2846</td>
</tr>
</tbody>
</table>

Table 6-8. Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Vender</th>
<th>Catalog</th>
</tr>
</thead>
<tbody>
<tr>
<td>basal medium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>glucose paper (various types)</td>
<td>Aldrich</td>
<td></td>
</tr>
<tr>
<td>Whatman Filter Paper (No. 1)</td>
<td>Fisher Scientific</td>
<td>09-805-1A</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity (per liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>200 mg</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>100 mg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>55 mg</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>200 mg</td>
</tr>
<tr>
<td>Resazurin</td>
<td>1 mg</td>
</tr>
<tr>
<td>FeCl₂·4H₂O</td>
<td>100 mg</td>
</tr>
<tr>
<td>Trace Metals Solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>Na₂S·9H₂O</td>
<td>500 mg</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>6 g</td>
</tr>
</tbody>
</table>

The first six compounds are added to distilled-deionized water, then purged with N₂ until solution turns from blue to pink. The remaining components are added, followed by a 15-minute purge with the 70% N₂/30% CO₂ gas mixture.

Table 6-10. Trace metals for anaerobic growth (DiStefano 1992).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCl₂·4H₂O</td>
<td>100</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>170</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>100</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>251</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>19</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</td>
<td>50</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>20</td>
</tr>
</tbody>
</table>