# Anaerobic Digestion - Introduction to Toxicity Curve and Feedback Inhibition

## INTRODUCTION

A number of organic and inorganic chemicals are toxic to methane-bacteria. The toxicity of these chemicals is dependent upon the present concentrations. Additionally, this toxicity is related to the presence/absence of other toxic chemicals, operating conditions, and the adaptability of the bacteria. Toxic outcomes are also dependent on whether exposures are chronic or acute. A basic toxicity curve can be used to determine the doses at which a given chemical imposes toxic effects.

Heavy metals have potential to attach to fibrils on the cell wall, entering the cell there and causing toxicity within. Oxygen and other electron acceptors also wreak havoc within anaerobic digesters, for obvious reasons. Benzene containing chemicals, found in many fruits, can cause methane-bacteria to slow metabolically.

Operating conditions, such as pH, can cause ammonia, hydrogen sulfate, and cyanide to become more noxious. In the ionized forms these chemicals cannot enter the cell, however non-ionized forms can enter the cell and affect bacterial enzymes. In these cases a feedback inhibition loop is used. The fatty acid intermediates of anaerobic digestion causes decreased pH in the system therefore ionizing many of the aforementioned chemicals.

## TASK(S)

<table>
<thead>
<tr>
<th>Activity 1 (30 minutes)</th>
<th>Digestion prep and set up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity 2 (50 minutes- 7 to 10 days following Activity 1)</td>
<td>Digestion analysis</td>
</tr>
<tr>
<td>Activity 3 (50 minutes)</td>
<td>Create a mode</td>
</tr>
</tbody>
</table>
## Anaerobic Digestion- Introduction to Toxicity Curve and Feedback Inhibition

### ACTIVITY/PROCESS

#### Digestion Prep and Set Up
- Separate into groups of 2-3 students
- Set water baths to 35 and 55 centigrade
- Obtain 3 plastic bottles for digestion vessels per group. Label as the following treatments: untreated, 1mL, 10mL, and 100mL
- Record weight of all empty vials. Add approximately 100mL sludge from onsite digester, cap and record weight.
- Add approximately 50g of homogenous waste substrate (collected from home, lunch, coffee grounds, etc). Cap and record total weight
- To the vessel labeled 1mL, add approximately 1mL of ammonia, cap and record weight
- To the vessel labeled 10mL, add approximately 10mL of ammonia, cap and record weight
- To the vessel labeled 100mL, add approximately 100mL of ammonia, cap and record weight
- Construct and record hypothesis as to which treatment will produce more biogas, and another as to which will produce a higher percentage of methane within the biogas
- Place digesters in water baths for 7-10 days, recording water bath temperature daily

#### Digestion Analysis
- Weigh each vessel
- Degas (unscrew cap, but do not remove)
- Weigh each degassed vessel
- Measure methane using gas sniffer (E Instruments) and carbon dioxide content using LabQuest (Vernier)
- Measure pH, temperature, and ammonia concentration of digestate, using LabQuest (Vernier)
- Create data table, bar chart, and perform class-wide ANOVA analysis using Microsoft Excel
- Discuss conclusions in terms of recorded hypotheses

#### Create a Model
- Using what you’ve read, and any additional resources, create a model drawing of the feedback inhibition loop demonstrated in anaerobic digestion. Be sure to include concepts such as volatile fatty acids, pH, hydrogen release, acetic acid formation, inhibition of methane-forming bacteria, toxicity, propionate, and decrease alkalinity.
- Choose at least one other feedback inhibition loop seen in the environment about which to write a 1-page description.
Anaerobic Digestion- Introduction to Toxicity Curve and Feedback Inhibition

**RESOURCES**


**ASSESSMENT**

- Identify strengths and weaknesses in data collection, graphing, and statistical analyses
- Discuss relationship among dose and toxicity
- Using data collect, write brief review toxicity of ammonia in terms of biogas and methane production. Reviews should incorporate data charts.
- Create and discuss feedback inhibition in nature.