Module 3:
Factors that affect AD

3.1: Microbial populations

3.2: Feedstock basics

3.3: Loading rate & retention times

3.4: Temperature & mixing

3.5: Environmental factors

3.6: Symptoms & seven causes of unstable AD

This curriculum is adapted from: eXtension Course 3: AD, University of Wisconsin

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Microbial populations

Bacteria & methanogens

Fermenting bacteria: bacteria that degrade organic compounds to organic acids like acetic acid.

- **Hydrolytic bacteria:** convert complex organics like polysaccharides and proteins to simpler molecules.
- **Acidogenic bacteria:** reduce simple organic molecules to organic acids.
- **Acetogenic**
- **Homoacetogenic**
- **Syntrophic**

→ convert organic acids to acetate

Methanogens: bacteria that convert acetic acid into methane.

Manure should provide all of the bacteria needed for anaerobic digestion.

Five functional groups of bacteria

1. **Fermenting bacteria**

2. **Hydrogen (H_2)-producing bacteria**

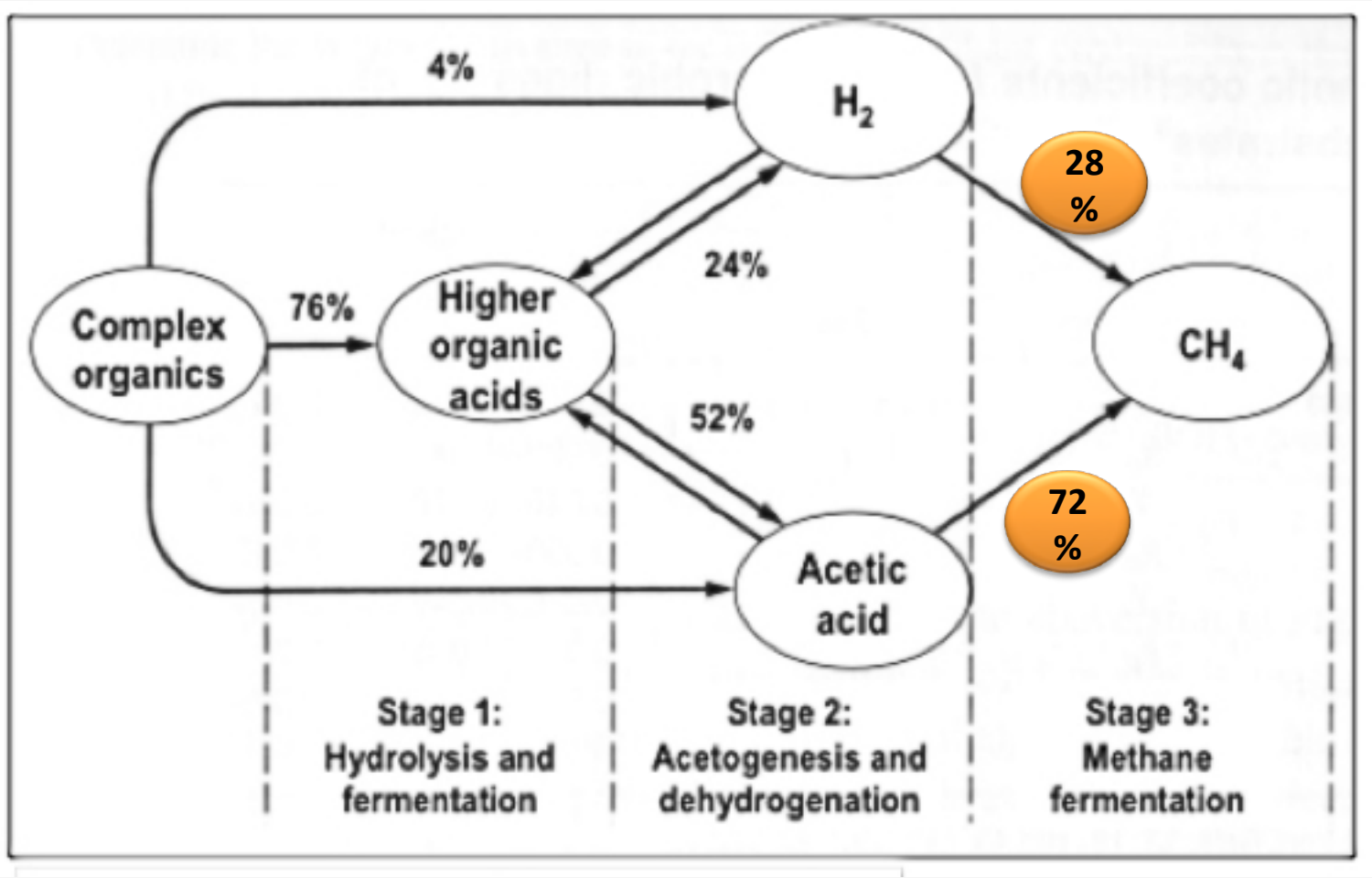
3. **H_2-consuming bacteria**

4. **CO_2-reducing methanogens** $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
   - ~20 – 3 0% methane production

5. **Aceticlastic methanogens** $CH_3COOH \rightarrow CH_4 + CO_2$
   - ~ 70% methane production

3 steps of AD

Most methane is made from **acetate,** but some is made by **reducing CO_2 with H_2.**



3 steps of AD

Stage 1: Complex organic molecules are broken into simple organic acids by bacterial enzymes secreted from (outside of) the bacterial cells. The process is **slow**, although the bacteria reproduce quickly.

Stage 2: Organic acids are converted to volatile fatty acids (VFAs), mainly acetic acid. This process is **rapid**! + carbon dioxide to methane.

Stage 3: Two populations of methanogens convert acetate to methane. This is usually the **rate-limiting step** because methanogens are slow-growing and sensitive to environmental factors.

1st population converts acetic acid to methane.

2nd population converts hydrogen + carbon dioxide to methane.

WPCF (1987)

Balancing fermenters & methanogens

Efficient AD occurs when bacterial populations are high and the activities of fermenting and methanogenic bacteria are balanced. Any change in AD conditions (like temperature & pH) affects that balance.

A 'sour' digester is an example of imbalance:

- Methanogens work between pH 6.8 – 7.2.
- Acetogenic bacteria don't mind low pH and keep making acetate.
- Increased acetate concentrations lower the pH.
- Methanogens die and acetate accumulates, pH stays low.
- Methane production stops.

The cure?

- Simply feed a plug flow AD.
- Stop feeding a mixed AD and wait for biogas production to resume.
- Test the slurry and treat as necessary (raise pH).
- As a last resort, empty it and start over.

AD terms to know

Stabilization: reduction of the volatile solids content by AD

Volatile solids: the portion of total solids that can be converted to biogas by AD

Total organic solids: all organic matter

Feedstock: any organic matter fed to the AD

Retention time: the time feedstock spends in the AD; length of process

Factors that affect AD

Physical factors:

- Temperature
- Hydraulic retention time (HRT)
- Solids retention time (SRT)
- Organic loading rate (OLR)
- Volatile solids loading rate
- Mixing

Chemical factors:

- pH
- Alkalinity
- Volatile fatty acids (VFAs)
- Nutrients
- Trace elements
- Toxins

Assessment!

Please answer the questions in **section 3.1** of the Module 3 Assessment.

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Feedstock basics

Examples of feedstock

Manure: has low energy since the feed has already been digested. But:

- Manure has a neutral pH & high buffering capacity (alkalinity)
- Has all the microbes needed for AD
- Has all the macro- & micronutrients needed for AD
- Manure is abundant & pumpable

Non-manure feedstock is often acidic and is balanced, and inoculated, when mixed with manure. It contributes energy & biogas production.

- Waste feed
- Food residuals
- Fats, oils & grease (FOG)
- Energy crops
- Waste from ethanol or biodiesel production
- Produce waste
- Cafeteria waste
- Farm animal mortalities

Feedstock selection may be regulated

Some states allow AD of animal mortalities or slaughterhouse waste but some don't. AD operators must check to be sure that they are complying with **federal and state regulations.**

Some states **regulate the amounts** of high-strength organics like ethanol syrup or FOG to a maximum amount.

Other cautions:

- Don't overload with **high-energy** feedstock like food waste.
- Don't feed **known toxins** like fossil fuel derivatives, ammonia or sulfides at high pH.
- **Recalcitrant** (or poorly degradable) material requires long retention times in order to degrade most of the VS.
- **Inert materials** yield headaches rather than biogas.

Vermont: who regulates what?

We'll cover feedstock regulation in detail in another module, but Vermont has two agencies with regulatory power that affects feedstock.

Vermont Agency of Natural Resources

- **Wastewater Division** grants indirect discharge permits to the **generators** of wastewater and liquid food processing materials.
- **Solid Waste Division** permit those **accepting** solid waste.
    - A full solid waste certification is required for AD facilities that accept solid pre- and post-consumer food residuals, whether they are collected in a clean stream or with municipal solid waste.

Vermont Agency of Food, Farms and Markets

- Requires on-farm digesters to report all feedstocks accepted via LFO, MFO or coming SMO (small farm operations) regulations.
- Blocks sale of separated solid bedding to other farms if AD facilities take any organic residuals including beef.

Vermont: not yet regulated

In Vermont, there are some organic residuals that are not yet regulated by ANR. These include:

- **Glycerol** (aka glycerin): the by-product of biodiesel production from FOG

- **Grease trap waste:** dilute FOG collected from wash water in restaurants
  - It's critical that GTW from industrial facilities, or containing heavy metals, or other chemicals toxic to the AD process be avoided.
  - Note that some GTW is thickened by the addition of flocculants. Some flocculants are biodegradable and non-toxic, but others are toxic to aquatic ecosystems and must be avoided.

C:N ratio

Anaerobic bacteria use **C for energy** and **N for building cells**.

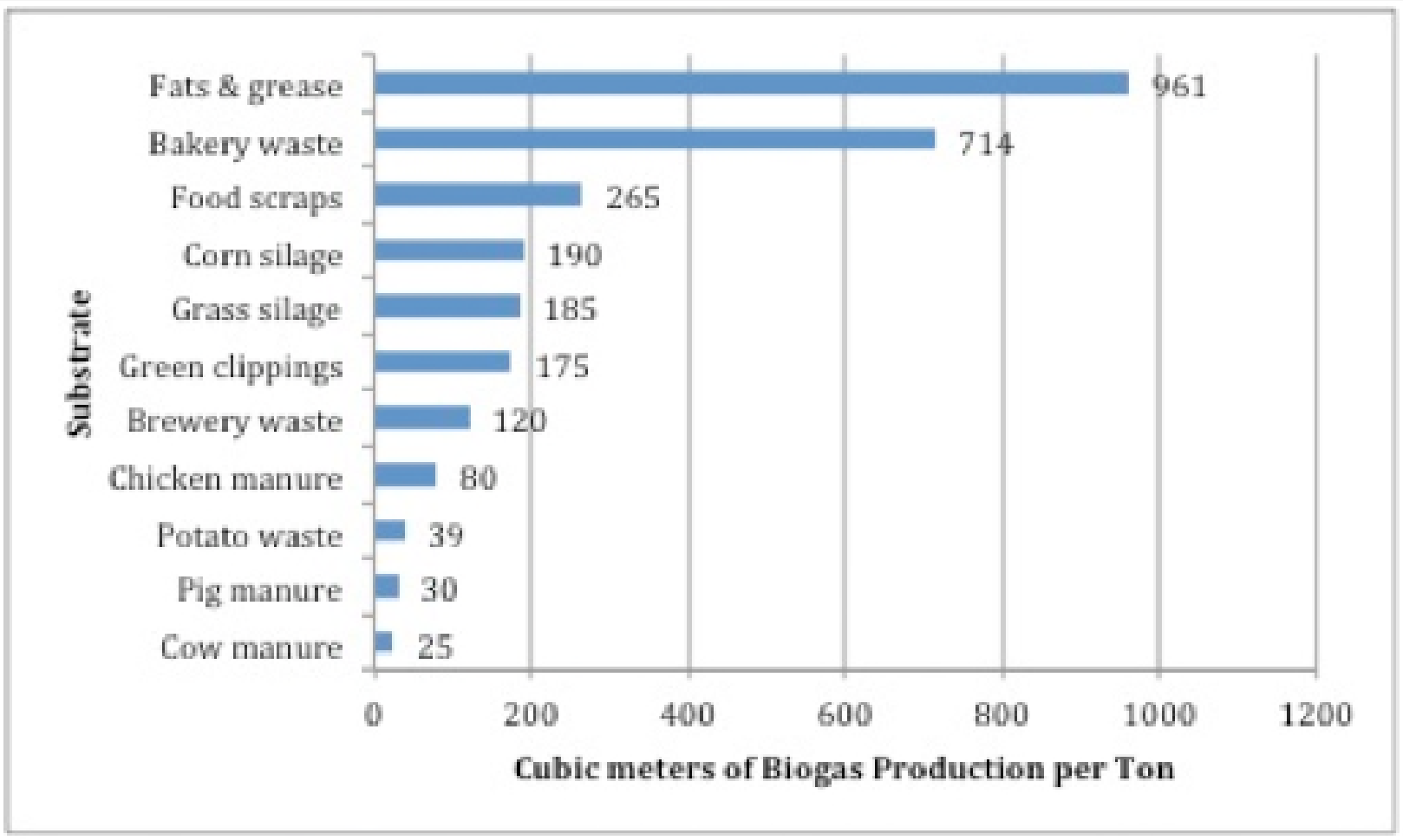
- Carbon is used 30-times faster than nitrogen so a **30:1 ratio** is optimal for AD.

- At **higher** C:N ratios the N is used up first & gas production then slows.

- At **lower** C:N ratios the C is used up and fermentation stops.
  - Lack of acetate then stops biogas production.
  - And excess N becomes excess ammonia.

Volatile solids

Volatile solids are the organic compounds that can be made into methane. The best feedstock materials have high levels of volatile solids.



Assessment!

Please answer the questions in **section 3.2** of the Module 3 Assessment.

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Loading rate & retention times

Feedstock loading

AD operators monitor and control AD feeding rates, **aka organic loading rates.**

Critical factors include:

- Concentration of feedstock (mas/volume)
- Volatile solids content of feedstock
- Inorganic (or inert) content of feedstock
- Volatile solids / AD volume
- Hydraulic retention time

Calculating the loading rate as VS

Example: complete mixed AD (50' dia x 20' deep w/ 5' cone depth)

- Fed 5,000 gallons manure/day @ 100F
- 6.5% TS, 69% VS, density =1

<u>Calculating manure volume</u>

cylinder = $(\pi)(r^2)(h) = (\pi)(25^2)(20) = 39{,}250\ ft^3$

cone = $(1/3)(r^2)(h) = (1/3)(25^2)(5) = 3{,}217\ ft^3$

total = $42{,}521\ ft^3$

<u>Calculating loading rate</u>

pounds TS/day = (gallons/day)(8.34 lb/gallon)(%TS)

= (5000)(8.34)(0.065) = 2,710 lb TS/day

pounds VS/day = (lb TS/day)(%VS) = (2,710 lb TS/day)(0.69) = 1,869 lb VS/day

loading rate = (lb VS/day) / volume of manure = 1,869 lb/day / $45{,}521\ ft^3$

= 0.04 lb / day / ft^3

Average loading rates are 0.02 – 0.37 lb VS / ft^3 volume

Hydraulic retention time (HRT)

Hydraulic retention time (HRT) (aka hydraulic loading): average days that feedstock stays in AD.

- Allows the bacteria enough time to convert all VS to methane.
- Related to AD capacity.
- HRT = AD volume (gallons) / feed volume (gallons/day).

Minimum HRT depends on:

- AD type and design;
- Temperature; and
- Type and volume of feedstock.

Tools to estimate biogas yield

These tools can be downloaded at no charge:

AgSTAR

- AgSTAR Handbook
- FarmWare

Unversity of Minnesota Extension

- Anaerobic Digester Economics Excel spreadsheet

Here at Vermont Tech we are estimating biogas & power production using feedstock energy values, expressed as volume of feedstock / ton of material), and historical data from our facility

Assessment!

Please answer the questions in **section 3.3** of the Module 3 Assessment.

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This curriculum is adapted from: eXtension Course 3: AD, University of Wisconsin

Temperature & mixing

Temperature

AD is possible at three temperature ranges:

AD	°C	°F	HRT
psychrophilic	20 - 25	68 – 77	up to 100 days
mesophilic	20 - 40	95 - 100	5 – 50 days
thermophilic	40 - 110	130 - 145	5 – 12 days

Higher temperatures:

- Speed up the AD process;
- Increase destruction of pathogens (thermophilic vs. mesophilic); and
- May allow digestion of **some refractory feedstock**: organic materials that resists degradation by AD.

Psychrophilic AD:

- Uses less parasitic energy for heating tanks, but requires larger tanks; &
- May reduce the level of human pathogens that require higher temperatures to thrive & grow.

Mixing methods

Biogas mixing: as biogas forms, bubbles rise to the surface = natural mixing

- Requires a loading rate of 0.4 lb VS / ft^3 / day.
- Heating causes convention currents that also provide some mixing.

Mechanical mixing: using impellers and pumps

- **Impellers** are blades attached to a shaft and motor; speed varies.
- **Pumps** should be strong enough to move the entire AD volume.
- Placing motors outside of AD tanks allows for easier servicing.

Mixing speed: mixing should be done as little as needed

- Studies show that start-up is best with slow mixing while faster mixing later improves long-term stability.

Note that plug-flow digesters are mixed **"zonally"** within each plug by vertical recirculation (bubbling) of biogas.

- www.dvoinc.net

Assessment!

Please answer the questions in **section 3.4** of the Module 3 Assessment.

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Environmental factors

Environmental factors can affect AD

A number of **environmental factors** affect AD:

- Presence of oxygen
- Temperature
- AD robustness
- pH range
- buffers
- VFA production
- Toxic materials
  - Alkaline / alkaline earth toxicity
  - Heavy metals
  - Sulfide toxicity
  - Ammonia toxicity

These can all be considered critical **operational parameters** and should be monitored to establish a baseline for robust AD operation.

Presence of oxygen

AD cannot occur in the presence of oxygen gas because even small amounts kills methanogens.

However, **small amounts of oxygen** are sometimes introduced into the gas space in order to oxidize, and therefore precipitate, sulfur.

- This technique is discussed in more detail in another module.

Digester robustness

Robust AD design and construction allows systems to handle changes of season and temperature.

AD systems can adapt to changes in temperature if they occur **gradually**.

Being able to heat organic material before feeding is helpful. If heating is not possible, **cold feedstock should be limited to <5% of the daily load**.

During our first year of operation Vermont Tech's facility (VTCAD) did not heat feedstock. Feeding the full feed volume of 16,000 gallons / day sometimes lowered temperatures in the hydrolysis tank for part of the day. However, the temperature in the more sensitive AD tank were not affected.

- When 70% full, VTCAD's hydrolysis tank has a volume of 73,500 gallons.
- The 16,000 gallons feed volume represents 21.7% of hydrolysis volume,
- …or 5% of AD volume.

pH range

The groups of bacteria responsible for AD **have different pH preferences**:

- Fermenting bacteria (hydrolysis & acetogenesis) perform best at pH **4.5 – 5.5** but will function above this range
- Methanogens don't function below pH 6, and perform optimally from **6.8 – 7.2** (though 6.4 to 8.0 can be tolerated)
  - Below pH 6, unionized VFAs are toxic to methanogens.
  - Above pH 8, unionized dissolved ammonia is toxic to methanogens.

pH measurements of slurry must be taken carefully and quickly because of high levels of dissolved CO_2. Biogas in the headspace is >30% CO_2, so the slurry gains quite a bit. The level of carbonates in the slurry helps determine pH. When samples are pulled some CO_2 evaporates quickly **causing pH to appear higher than it is in the AD**.

Saber (2009); Yadvika (2004); WPCF (1987)

Buffers

The pH of the slurry is determined by the balance of VFAs, CO_2 & **alkalinity**.

This is the **buffering capacity:** the ability of the slurry to resist changes of pH when chemical composition changes.

In AD systems, the **carbonate acid-base buffering** system exerts the most control over pH.

- In a balanced AD, VFA concentrations are low and total alkalinity should be roughly equal to bicarbonate alkalinity.
- Bicarbonate alkalinity should be 2500 – 5000 mg/L in a stable AD system.
- Bicarbonate buffer is present in feedstock, particularly manure, and…
- … are created by methanogens: carbonates, bicarbonates, ammonia.z

When pH begins to drop, buffering capacity is nearly depleted.

- The rate of fermentation is greater than the rate of methanogenesis.
- Bacteria may be growing slowly or have been washed out.
- Toxins may be present.

VFA:TA ratio

The ratio of volatile fatty acids : total alkalinity (**Ripley ratio**) is a useful test.

- pH a bit like starlight: pH values indicate biochemical balance from the past and is a good indicator of what has happened, rather than current state.
- The VFA:TA gives operators a better view of what is going on in the digester now. For example: the ratio can reach 7.5:1 before pH begins to change.
- Increasing the VFA concentration will increase biogas production & power output. But without buffering capacity (alkalinity) increasing VFA concentrations will lower pH & cause bacteria to stop functioning and/or die. This is often referred to as 'souring'.
- For manure, the VFA:TA should be no higher than 2:1.
- AD systems with low solids (< 3% TS) are more sensitive to changes in acidity, so use lower VFA:TA ratios.

Treatment for a sour digester?

1. Feed a plug flow AD or 'starve' a complete mix AD
2. Add buffers like Na_2CO_3, CaO, $CaCO_3$ to increase pH.

Production of volatile fatty acids

In a stable AD system, VFAs are used by methanogens as quickly as they are made & concentration of acetic acid in the slurry should be **50 – 300 mg/L**.

If the loading rate is increased or feedstock rich in volatile solid is suddenly added, production of VFAs will surge and pH will drop. Preliminary data from the colleges' digester shows that VFAs are liberated during the AD process, **peaking in the hydrolyzer**. VFA concentrations much reduced in effluent (98.1%), as VFAs have been converted to methane.

VTCAD samples (Dec 2014)	Total VFA (mg/L)
College manure	2884
Abdie manure	3687
Feedstock preparation pit	5400
Hydrolysate	11565
Digestate	411
Effluent	225

Nutrients

The **major nutrients** required for production of the bacteria that perform anaerobic digestion are phosphorous and nitrogen.

Smaller amounts of sodium, potassium, magnesium, chloride, and sulfate ions are also required.

Trace amounts of iron, copper, manganese, zinc, molybendum and vanadium are also required for microbial growth.

Generally, growth rates of microbes in anaerobic digestion are low, and sufficient nutrients are supplied in feedstock.

WPCF (1987)

Inhibition

The AD process can be inhibited by a number of factors: operational error or lack of fine tuning; or toxins present in feedstock.

Operational examples:

- Low temperature; over-stirring; …
- Feedback inhibition caused by build up of VFAs or H_2S.

Toxins:

Note that some toxins are always present in the AD process. They can be stimulatory (good) at low concentrations, tolerated at higher concentrations, and downright toxic above a specific threshold.

- Specific inorganic materials
- Specific organic materials.

Fortunately, bacteria – methanogens included – are capable of **adapting** to some amount of toxins. However, this adaptability makes it difficult to determine precise concentrations at which toxicity occurs.

Toxicity can be acute or chronic

Acute toxicity: rapid exposure of unacclimated bacteria to a relatively high concentration of toxin(s)

- The effect is sudden.

Chronic toxicity: gradual & long-term exposure of unacclimated bacteria to toxin(s)

- The effect builds over time.

Generally, toxicity depends on a number of **factors**:

1. Ability of bacteria to adapt to a constant concentration of the toxin;
2. Presence or absence of other toxins; &
3. Changes in operational conditions.

Acclimatization occurs by two means:

1. Bacteria repair damaged enzyme systems used to degrade the toxin.
2. Expansion of a population of bacteria that can degrade the toxin.

Either way, toxin levels cannot be high, and time is required.

Symptoms of toxicity

Symptoms of toxicity **may appear slowly or rapidly**, depending on the type of toxin, its concentration and operational conditions.

- Loss of hydrogen
- Loss of methane
- Loss of alkalinity and/or pH
- Increase of VFA concentration (increasing Ripley ratio)

Gerardi (2003)

Inorganic & organic AD toxins

- Alcohols (isopropanol)
- Alkaline cations (Ca^{+2}, Mg^{+2}, K^{+1}, Na^{+1})
- Alternate electron acceptors (NO_3^{-1} & SO_4^{-2})
- Ammonia*
- Benzene ring compounds
- Detergents (like dodecyl or lauryl sulfates)
- Food preservatives
- Chlorinated hydrocarbons
- Cyanide
- Disinfectants
- Formaldehyde (> 100 mg/L)
- Heavy metals*
- **Hydrogen sulfide***
- Organic nitrogen compounds (like acrylonitrile)
- Pharmaceuticals (like monensin)
- Solvents
- **VFAs and long-chain fatty acids**

* Most commonly described

feedback inhibition

Gerardi (2003)

Toxic values for some inorganics (1)

	Gerardi (mg/L)	WPCF (soluble mg/L)
Ammonia (NH_3)	1,500	
Arsenic (As)	1.6	
Boron (B)	2	
Cadmium (Cd)	0.02	
Chromium (Cr^{+6})	5 - 50	3.0
Chromium (Cr^{+3})	50 - 500	
Copper (Cu)	1 - 10	0.5
Cyanide (CN^{-1})	4	
Iron (Fe)	5	
Magnesium (Mg)	1,000	
Sodium (Na)	3,500	
Sulfide (S^{-1})	50	
Zinc (Zn)	5 - 20	1.0
Nickel (Ni)		2.0

Toxic values for some inorganics (2)

	simulatory (mg/L)	Moderate inhibition (mg/L)	Strong inhibition (mg/L)
Calcium (Ca^{+2})	100 – 200	2,500 – 4,500	8,000
Magnesium (Mg^{+2})	75 – 150	1,000 – 1,500	3,000
Potassium (K^{+1})	200 - 400	2,500 – 4,000	12,000
Sodium (Na^{+1})	100 – 200	3,500 – 5,500	8,000
Ammonium (NH_4^{+1})	50 – 200	1,500 – 3,000*	> 3,000

* At pH > 7.4 – 7.6

Toxic values from some organics

	toxic concentration (mg/L)
alcohol, allyl	100
alcohol, octyl	200
acrylonitrile	5
benzidine	5
chloroform	10 - 16
carbon tetrachloride	10 - 20
methylene chloride	100 - 500
1,1,1-trichloroethane	1
trichlorofluoromethane	20
trichlorotrifluoroethane	5

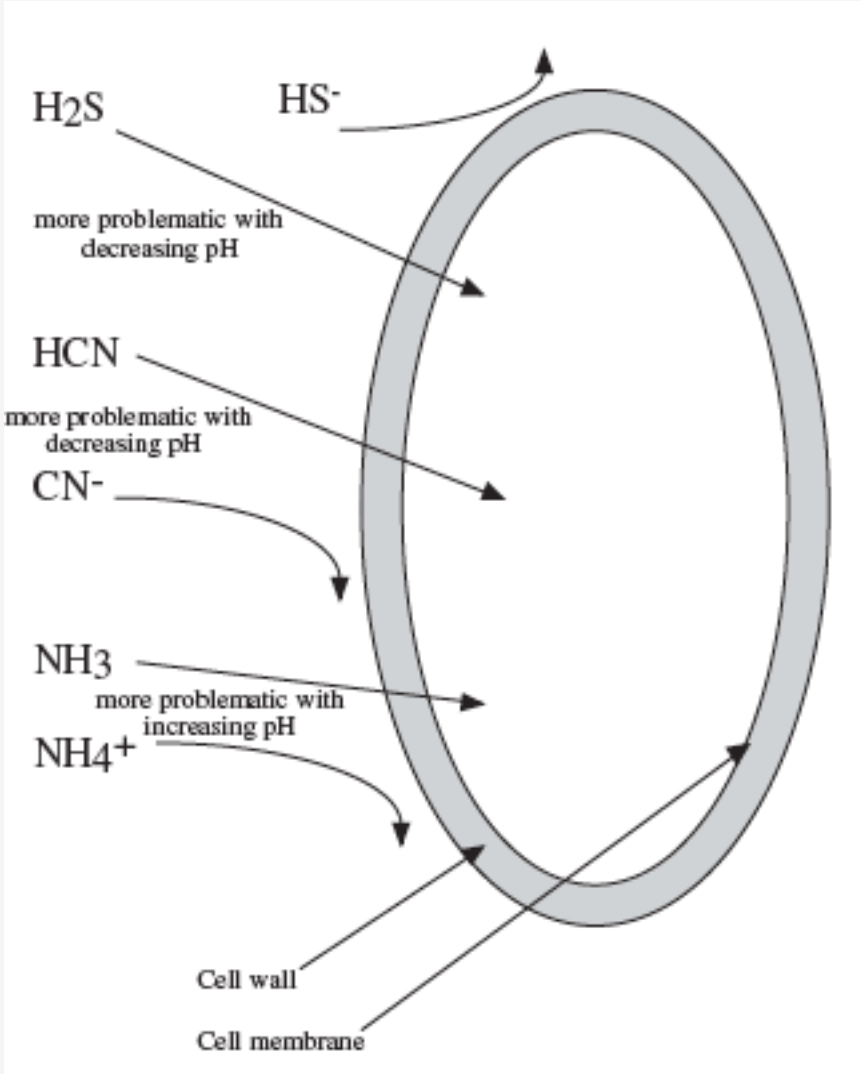
Gerardi (2003)

pH dependent toxins

The toxicity of ammonia (NH_3), sulfides (S^{-2}) & cyanides (CN^{-1}) is **pH dependent.**

Ammonia becomes less toxic as pH decreases because low pH adds a H to create ammonium. Ammonium can't cross the bacterial membrane as readily.

Sulfides and **cyanides** become more toxic as pH drops because their protonated & neutral forms (H_2S and HCN) are better able to cross bacterial membranes than non-protonated forms.



Gerardi (2003)

Ammonia toxicity

Ammonia nitrogen (NH_4^{+1}-N or ammonium ions NH_4^{+1}) are both reduced forms of ammonia and can be added in feedstock or produced during digestion of amino acids & proteins.

The form of ammonia depends on pH:

$$NH_4^{+1} \leftrightarrow NH_3 + H^{+1}$$

equal concentrations @ 9.3
only 0.5% ammonia @ 7.0

- (NH_3) is **toxic to methanogens** & toxicity is pH-dependent, decreasing with decreasing pH as ammonia is converted to ammonium (NH_4^{+1}).
- Unacclimated bacteria can be **inhibited by ammonia concentrations >50 mg/L**, but acclimated bacteria are more tolerant:

NH4+1 / NH3 (mg/L)	effect
50 - 200	stimulatory (good)
200 - 1000	no adverse effect
1500 – 3000	inhibitory at pH > 7 (can cause failure)

Ammonium is the preferred source of N for reproducing bacteria & buffers.

Gerardi (2003)

Ammonia toxicity: treatment

Treatment?

- Lower digester pH
- Dilute AD slurry with material less likely to form ammonia: low protein feedstock.

To some extent, ammonia toxicity is **'self-correcting':**

- Ammonia concentrations rise (perhaps due to increasing pH or high-protein feedstock);
- Methanogens become inhibited;
- Fermenting bacteria continue to produce VFAs;
- Accumulating VFAs lower pH, converting ammonia to ammonium; and
- This *may* allow methanogenesis to resume.

Gerardi (2003)

H_2S toxicity

Sulfides (S^{-2}) are an essential bacterial nutrient, but nearly never limiting.

- Excess sulfide or hydrogen sulfide (H_2S) are toxic
  - H_2S is an acid and a gas!
  - Amino acids & proteins are the most common sources of sulfur.

Sulfate (SO_4^{-2}) has little effect on methanogenesis, but this form of S is reduced to H_2S by sulfate-reducing bacteria (SRB).

H_2S toxicity is most severe for hydrogen-consuming methanogens & less severe fro acetoclastic methanogens. Fermenting bacteria that break feedstock down into small organic acids are also susceptible to inhibition by H_2S.

- Toxicity occurs at dissolved H_2S levels of **> 200 mg/L**.
- H_2S **partitions** between digester slurry and biogas (ie dissolved vs. gas).
- H_2S production **increases at low organic loading rates** because of decreased biogas production. Increased concentrations of CO_2, H_2 and CH_4 inhibit formation of H_2S.

H_2S toxicity: treatment

Precipitation:

Sulfides are toxic only in their **soluble** form. Sulfide can be **precipitated** (made solid rather than soluble) by reaction with metals, most commonly iron.

- Addition of iron ions (typically as **ferric or ferrous chloride**) precipitates sulfides, forming a black sludge.
- Note that iron precipitation lowers the concentration of sulfides and H_2S, but does not completely prevent H_2S formation. This treatment lowers [H_2S].

Diluting slurry to reduce the concentration of sulfides.

Feedstock: reduce the amount of sulfate & sulfide in feedstock, mainly by monitoring and capping levels of protein in the AD diet.

Scrubbing biogas to remove H_2S & **recirculating the scrubbed biogas** into the AD slurry so that the remaining methane, carbon dioxide and hydrogen can inhibit formation of H_2S.

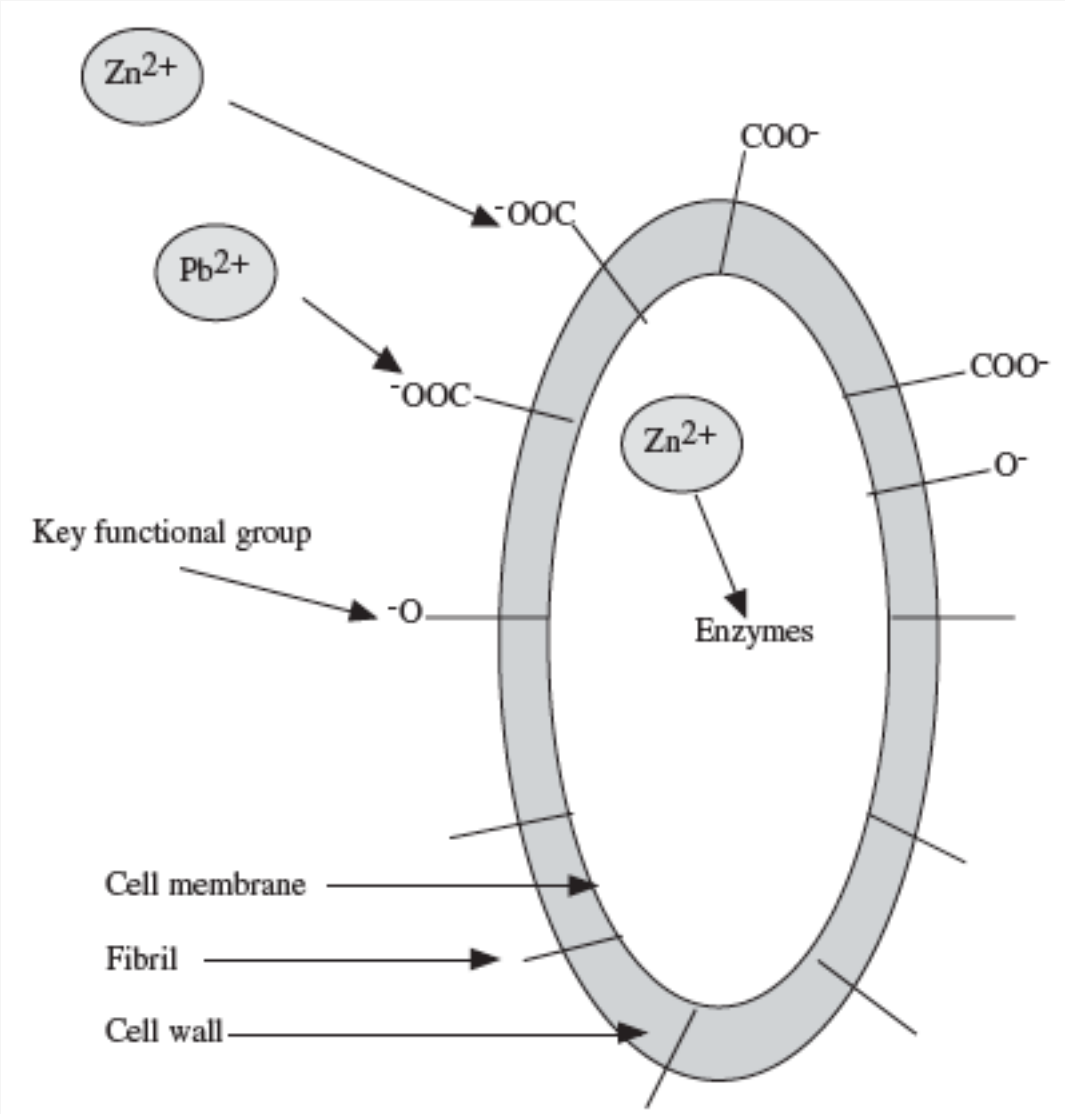
Gerardi (2003)

Heavy metal toxicity

Heavy metal ions inhibit methanogensis when present in **soluble** form: ionic & not combined with anions.

- Cobalt ion (Co)
- *Copper ion (Cu)
- Iron ion (Fe)
- *Nickel ion (Ni)
- *Zinc ion (Zn)

Metal ions absorb to anionic (negatively charged) functional groups on bacterial membranes. Once taken in, the ions inhibit enzyme activity & prevent methanogenesis.



Gerardi (2003)

Heavy metal toxicity: treatment

Avoid contaminated feedstock: feedstock with significant concentrations of heavy metal ions.

- Cu, Ni, Zn are the most problematic
- Cu is often used as in dairy farm footbaths.

Precipitate the metal ions by adding **chelating agents** that will bind them, reducing the concentration of ions that can bind to bacterial cells.

Chelating agents?

- Oxides
- Hydroxides
- Sulfides

] Precipitation increases at pH > 7.5

- Carbonates

Note that chloride and nitrate combine with metals but do not form insoluble precipitates.

Alternate electron acceptor toxicity

Nitrate (NO_3^{-1}) and **sulfate (SO_4^{-2})** ions can inhibit methanogenesis by increasing the redox value in the AD process.

- Methanogenesis requires very low redox values of -300 mV.
- When nitrate & sulfate are present, sulfate reducing bacteria (SRB) out-compete methane forming bacteria. H_2S concentrations rise and methane levels drop.

Treatment = avoidance

Alkaline cation toxicity

These metal ions are often added to AD to **increase alkalinity** and **buffer pH**.

- Ca^{+2}
- Mg^{+2}
- K^{+1}
- Na^{+1}

Alkaline cations can have **stimulatory or inhibitory effects**, depending on their concentration.

- At 100 – 400 mg/L AD activity is enhanced.
- At concentration > 1500 mg/L significant toxicity can occur.

Treatment = dilution or avoidance in feedstock.

Benzene ring compound toxicity

Methanogenesis is inhibited by a number of compounds whose structure is based on **benzene** or **phenolic rings**.

- Benzene
- Pentachlorophenol
- Phenol
- Phenolic compounds
- Toluene

- Chlorophenols
- Nitrophenols
- **Tannins**

From fruits & vegetables & coffee

- Toxic at 700 mg/L

Treatment = avoidance of toxic levels

Feedback inhibition

Fermentation produces 'intermediate' compounds that methanogens convert to methane:

- H_2 gas
- VFAs

At high concentrations, these intermediates cause **feedback inhibition** in order to slow down production until conversion can catch up.

- Feedback inhibition slows the metabolic rate of fermenting bacteria…
- … but also inhibits production of methane.

High partial pressure (concentration) of H_2 inhibits acetate-producing bacteria.
Accumulation of VFAs inhibits methanogens by direct toxicity:

- Increased **propionate** concentration is a sign of excess VFAs.
- **Loss of alkalinity** or **decrease in pH** is caused by VFA accumulation.

Two-phase AD (separation of hydrolysis & AD as in VTCAD's design) usually decreases feedback inhibition, increases stability & resistance to toxins.

VFA toxicity

Build up of **1-3 carbon VFAs** in unionzed form decreases alkalinity and pH.

- Toxicity occurs at neutral pH.
- Both acid-forming and methane-forming bacteria are inhibited.
- **Propionate** is the most toxic VFA.
- Toxic effects occur at propionate concentrations of < 5 mg/L.

VFA	aka	number of carbons
Acetic acid	acetate	2
Propionoic* acid	propionate	3
Butyric acid	butyrate	4
Valeric acid	valerate	5

Treatment:

Overcome VFA inhibition by add alkaline compounds to buffer pH.

Long-chain fatty acid toxicity

Long-chain fatty acids: the structure of these fatty acids is very similar to the lipids found in the cell walls of acetoclastic and methane-forming bacteria, causing these fatty acids to **dissolve the cell wall** and kill bacteria at low concentrations.

- This is an example of the 'like dissolves like' principle of chemical solubility.

Fatty acid	Carbons	Sat/unsat	formula
Caprylic (octanoic)	8	saturated	$CH_3(CH_2)_6COOH$
Capric (decanoic)	10	saturated	$CH_3(CH_2)_8COOH$
Lauric* (dodecanoic)	12	saturated	$CH_3(CH_2)_{10}COOH$
Myristic (tetradecanoic)	14	saturated	$CH_3(CH_2)_{12}COOH$
Oleic (cis-9-octadecanoic)	18	unsaturated	$CH_3(CH_2)_7=CH(CH_2)_7COOH$

- All FAs of 8 – 18 C can be toxic.
- **Lauric** is the most toxic.
- Combinations can be synergistically toxic.
- Concentrations of **> 500 g/L** can be toxic to AD.

Gerardi (2003)

Assessment!

Please answer the questions in **section 3.5** of the Module 3 Assessment.

Module 3:
Factors that affect AD

3.1: Microbial populations

3.2: Feedstock basics

3.3: Loading rate & retention times

3.4: Temperature & mixing

3.5: Environmental factors

3.6: Symptoms & seven causes of unstable AD

This curriculum is adapted from: eXtension Course 3: AD, University of Wisconsin

Symptoms & causes of unstable AD

Symptoms of unstable AD

Indicators of instability tend to be increases or decreases in operational parameters:

Decreased:

- Biogas production
- Methane production
- Alkalinity
- pH
- Destruction of volatile solids (VS)

Increased:

- VFA concentration
- % CO2 in biogas

Methane is a better indicator than biogas volume.

- Changes in feedstock (drop in volume of VS content) normally decrease biogas & methane production.

Drops in methane + alkalinity are significant indicators of methanogen toxicity.

Drop in methane (but not alkalinity) indicates that both fermenters & methanogens are inhibited.

7 causes of unstable (upset) AD

Interruptions in steady state conditions cause upset & unstable AD. We can boil down the causes to **seven basic conditions:**

1. Hydraulic overload
2. Organic overload
3. pH change
4. Temperature fluctuation
5. Toxicity
6. Large purge of sludge
7. Sudden changes

1. Hydraulic overload

Hydraulic overload: when the HRT is reduced too much, so that methanogens are unable to reproduce fast enough to replace bacteria lost to washout (removal along with effluent).

- Overfeeding
- Purging sludge
- Reduction in digester volume

Symptoms of hydraulic overload:

- Loss of alkalinity
- Build up of organic acids (sour digester)
- Drop in digester temperature
- Decreased methane production
- Decreased VS destruction

2. **Organic overload:** when the organic loading rate is too high.

   Symptoms of organic overload:
   - Overfeeding high protein feedstock causes build up of ammonia.

3. **pH** of less than **6.8** in the AD tank is a sign of process instability.
   - Loss of alkalinity is usually seen before the drop in pH, so monitoring of alkalinity is more useful.

4. Significant changes in the **temperature** of the AD tank can be caused by feeding too much material at one time.
   - In a two-stage system, feeding may transiently decrease the temperature of the hydrolysis tank, but should not affect the AD tank.

5. **Toxicity** …. We've covered that!

6. **Large purges of slurry** lowers the HRT and may lower temperature.

7. **Sudden changes in feedstock or operational process** can shock or overwhelm microbes before they have a chance to adapt.

 Gerardi (2003)

Assessment!

Please answer the questions in **section 3.6** of the Module 3 Assessment.

Sources & resources

This curriculum is a modification of the wonderful:

- eXtension Course 3: AD, University of Wisconsin

http://fyi.uwex.edu/biotrainingcenter/online-modules/series-three-anaerobic-digestion/

Additional sources & resources:

Fry (1973)

Gerardi, M.H. (2003) The Microbiology of Anaerobic Digestion, John Wiley & Sons, New Jersey, ISBN 0-471-20693-8

Saber (2009) Technology Investigation, Assessment, and Analysis http://media.godashboard.com//gti/Pipeline_Quality_Biomethane_FINAL_TASK_1_REPORT2.pdf

Yadvika, S., Sreekrishnan, T., Kohli, S., Rana, V. (2004) Enhancement of biogas production from solid substrates using different techniques – a review, Bioresources Technology, 95: 1-10