# ENVE 424
# Anaerobic Treatment

## Lecture 6
## Toxic substances in anaerobic treatment

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# Basic Fundamentals

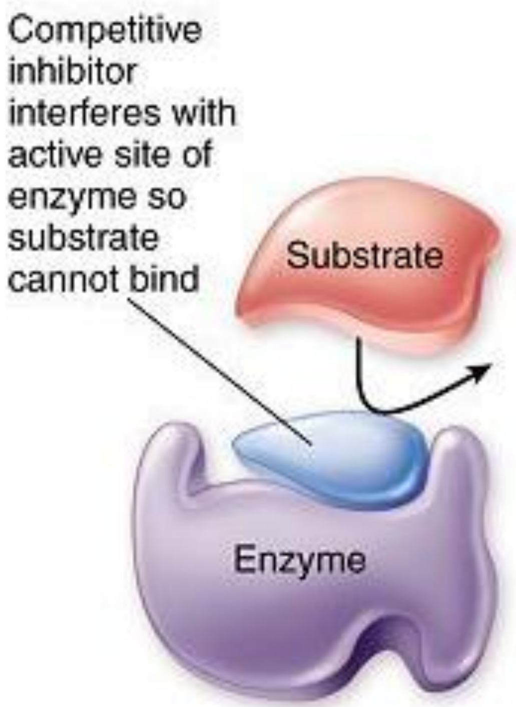
- Inhibition
  - Competitive Inhibition
  - Uncompetitive Inhibition
  - Noncompetitive Inhibition
- Toxicity

# Inhibition – Competitive Inhibition

- The inhibitor binds to the active site and prevents binding of the substrate
- Inhibitor and the substrate are chemically similar

$$[E]+[S]\overset{K_S}{\longleftrightarrow}[E\cdot S]\overset{k_2}{\longrightarrow}[E]+[P]$$

$$[E]+[I]\overset{K_I}{\longleftrightarrow}[E\cdot I]$$

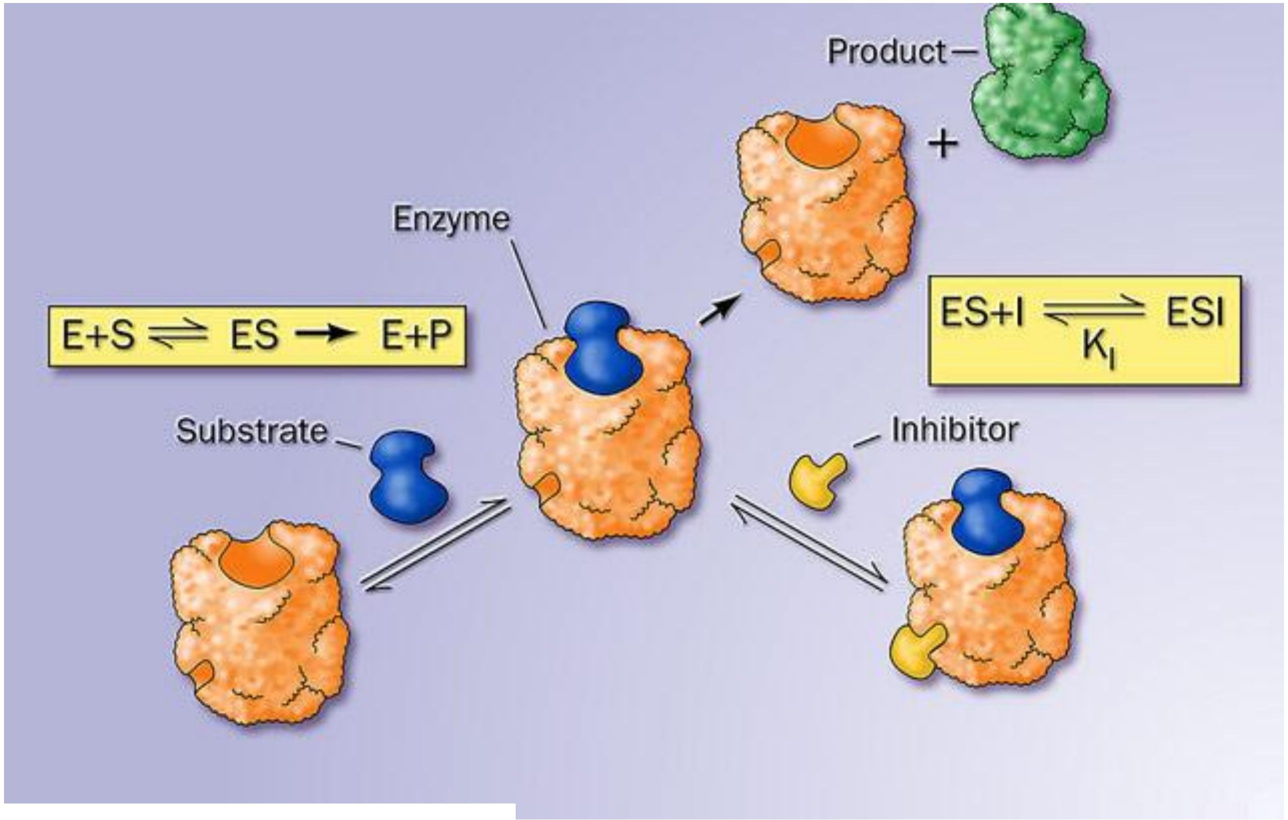



(a) Competitive inhibition

Ref:http://karimedalla.wordpress.com/2012/10/17/7-6b-enzymes/

# Inhibition – Uncompetitive Inhibition

- The inhibitor can only bind to Enzyme/Substrate complex
- Inhibitor and the substrate **<u>are not</u>** chemically similar




Ref:http://www.chemistrypictures.org/index.php/enzymes/4Uncompetitive_inhibition

# Inhibition – Noncompetitive Inhibition

- The inhibitor binds alters the active site of the enzyme
- Inhibitor can bind to either free enzyme or Enzyme/Substrate complex and prevents product formation
- Inhibitor and the substrate **<u>are not</u>** chemically similar

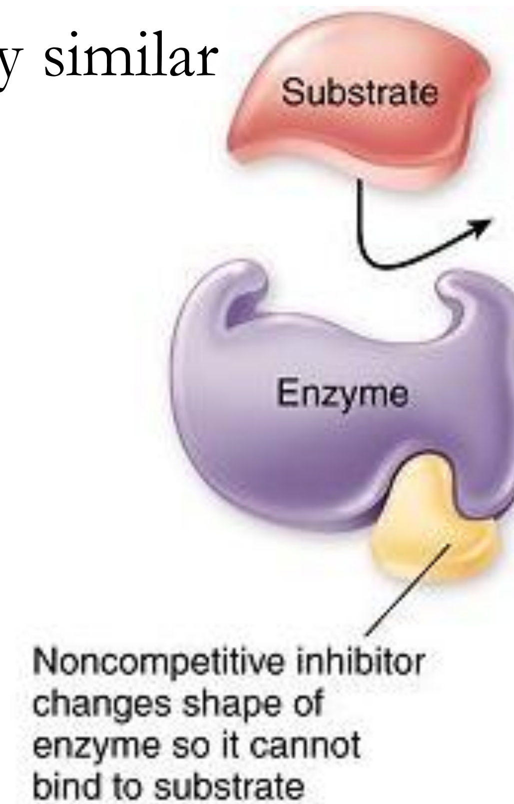
$$[E]+[S] \xleftrightarrow{K_S} [E \cdot S] \xrightarrow{k_2} [E]+[P]$$

$$+ \qquad\qquad\qquad\qquad +$$

$$[I] \qquad\qquad\qquad\qquad [I]$$

$$\Updownarrow K_I \qquad\qquad\qquad\qquad \Updownarrow K_I$$

$$[E \cdot I]+[S] \xleftrightarrow{K_S} [E \cdot S \cdot I]$$



(b) Noncompetitive inhibition

Ref:http://karimedalla.wordpress.com/2012/10/17/7-6b-enzymes/

# Toxicity vs. Inhibition

- Inhibition
  - Binding of an enzyme is reversible
  - Once bound inhibitor is released from the enzyme, enzyme will function again
- Toxicity
  - Irreversible
  - If the enzyme has been rendered by the binding of the inhibitor or if the bound inhibitor does not release

# Basic Fundamentals

1) For any material to be inhibitory or toxic, it must be in solution (soluble as opposed to particulate form)
   - If the substance is not in solution, it cannot pass through the cell wall
2) Toxicity is a relative term
   - Many inorganic or organic soluble materials can be either stimulatory or inhibitory or toxic

3) Acclimation

- When the concentration of potential inhibitory/toxic materials are slowly increased, some organisms can rearrange their metabolic resources
- Under shock load conditions sufficient time is not enough to achieve acclimation

# Basic Fundamentals

## 4) Antagonism and Synergism

- Antagonism: Reduction of the toxic effect of one substance by the presence of another substance
- Synergism: Increase of the toxic effect of one substance by the presence of another substance

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# Toxicity in AD

- A variety of inorganic and organic wastes can cause toxicity in anaerobic digesters.
- Many toxic wastes are removed in primary clarifiers and transferred directly to the anaerobic digester.
- Heavy metals may be precipitated as hydroxides in primary sludge.
- Organic compounds such as oils and chloroform are removed in primary scum & sludge.
- Industrial wastewaters often contain wastes that are toxic to anaerobic digesters.

# Toxicity in AD

- Toxic substances causing inibition on AD may be;
  - Components of the influent waste stream or
  - By-products of the anaerobic microorganims
- Although ranges of values exist at which toxicity occurs for specific organic and inorganic compounds, anaerobic bacteria and methanogens often can tolerate higher values by acclimating to toxic substances.

# Toxicity threshold

Toxicity threshold of a substance is determined by several factors including;

- The ability of the bacteria to adapt to a constant concentration of the toxic substance,
- Absence or presence of other toxic wastes, and
- Changes in operational conditions.

# Acute and chronic toxicity

**Toxicity**

**<u>Acute</u>**

Rapid exposure of an unacclimated population to relatively high concentrations of a toxic waste

**<u>Chronic</u>**

Gradual and relatively long exposure of an unacclimated population of bacteria to a toxic waste.

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# Chronic toxicity

- Anaerobic bacteria & methanogens may acclimate to chronic toxicity by two means;
  - They may repair damaged enzyme systems in order to adjust to the toxic wastes or degrade the toxic organic compound.
  - They may grow a relatively large population that is capable of developing the enzyme systems necessary to degrade the toxic organic compounds.

# Chronic toxicity

- Time of chronic toxicity in an anaerobic digester is determined by;
  1. The time of contact between the toxic waste and the bacteria
  2. The ratio of toxic waste to the bacterial population

# Toxic substances in AD

- Volatile Fatty Acids
- Sulfides
- Ammonia
- Heavy metals
- Alkaline metals ($Ca^{2+}$, $Mg^{2+}$, $K^{+}$ and $Na^{+}$)
- Cyanide
- Anthropogenic & recalcitrant compounds

# Toxic substances in AD

**TABLE 17.1 Inorganic and Organic Toxic Wastes to Anaerobic Digesters**

| |
|---|
| Alcohols (isopropanol) |
| Alkaline cations ($Ca^{2+}$, $Mg^{2+}$, $K^+$, and $Na^+$) |
| Alternate electron acceptors, nitrate ($NO_3^-$) and sulfate ($SO_4^{2-}$) |
| Ammonia |
| Benzene ring compounds |
| Cell bursting agent (lauryl sulfate) |
| Chemical inhibitors used as food preservatives |
| Chlorinated hydrocarbons |
| Cyanide |
| Detergents and disinfectants |
| Feedback inhibition |
| Food preservatives |
| Formaldehyde |
| Heavy metals |
| Hydrogen sulfide |
| Organic-nitrogen compounds (acrylonitrile) |
| Oxygen |
| Pharmaceuticals (monensin) |
| Solvents |
| Volatile acids and long-chain fatty acids |

Ref: Gerardi M.H. The microbiology of anaerobic digesters. Wiley Interscience. 2003

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# Toxic substances in AD

**TABLE 17.2 Toxic Values for Selected Inorganic Wastes**

| Waste | Concentration (mg/l) in Influent to Digester |
|---|---|
| Ammonia | 1500 |
| Arsenic | 1.6 |
| Boron | 2 |
| Cadmium | 0.02 |
| Chromium ($Cr^{6+}$) | 5–50 |
| Chromium ($Cr^{3+}$) | 50–500 |
| Copper | 1–10 |
| Cyanide | 4 |
| Iron | 5 |
| Magnesium | 1000 |
| Sodium | 3500 |
| Sulfide | 50 |
| Zinc | 5–20 |

**TABLE 17.3 Toxic Values for Selected Organic Wastes**

| Waste | Concentration (mg/l) in influent to digester |
|---|---|
| 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 |

Ref: Gerardi M.H. The microbiology of anaerobic digesters. Wiley Interscience. 2003

# Indicators of toxicity

- Disappearence of $H_2$
- Disappearence of $CH_4$
- Decreases in alkalinity and pH
- Increase in volatile fatty acid concentration

# Toxicity control measures

- Removal of toxic substances from the feed
- Dilution of the feed with another waste to bring toxic substance levels below toxic threshold value
- Addition of chemicals to form a non-toxic complex or insoluble precipitate
- Addition of an antagonistic substance.

# Ammonia toxicity

- Ammonium ($NH_4^+$), a reduced form of nitrogen may enter to an AD via influent or may be produced during hydrolysis of amino acids and proteins.
- Ammonia (base) combines with carbondioxide and water to form ammonium bicarbonate
- If the protein concentration is too high (slaughter house – urine) ammonia may reach to toxic levels.

# Ammonia toxicity

- Reduced nitogen exits in two forms, the ammonium ion ($NH_4^+$) and free or unionized ammonia ($NH_3$).
- $NH_4^+$ are used by anaerobic bacteria as a nutrient source for nitrogen & also provide a buffering capacity.
- $NH_3$ is the toxic form causing inhibition in anaerobic systems
- **Ammonia ($NH_3$ )** concentration of about 100 mg/L causes inhibition in acetate-fed anaerobic systems
- **$NH_4^+$ nitrogen** concentration found to cause inhibition aroung 3000 mg/L

# Ammonia toxicity

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

Free (unionized) ammonia

Ammonium ion

$$FA = \frac{TN}{1 + 10^{(pK_a - pH)}}$$

$$pK_a = 0.09018 + \frac{2729.92}{T + 273.15}$$

TN : Total $NH_3$-N, mg/l

FA : Free $NH_3$-N, mg/l

pKa : Dissociation constant for $NH_4^+$ (8.95 at 35°C)

T : Temperature, °C

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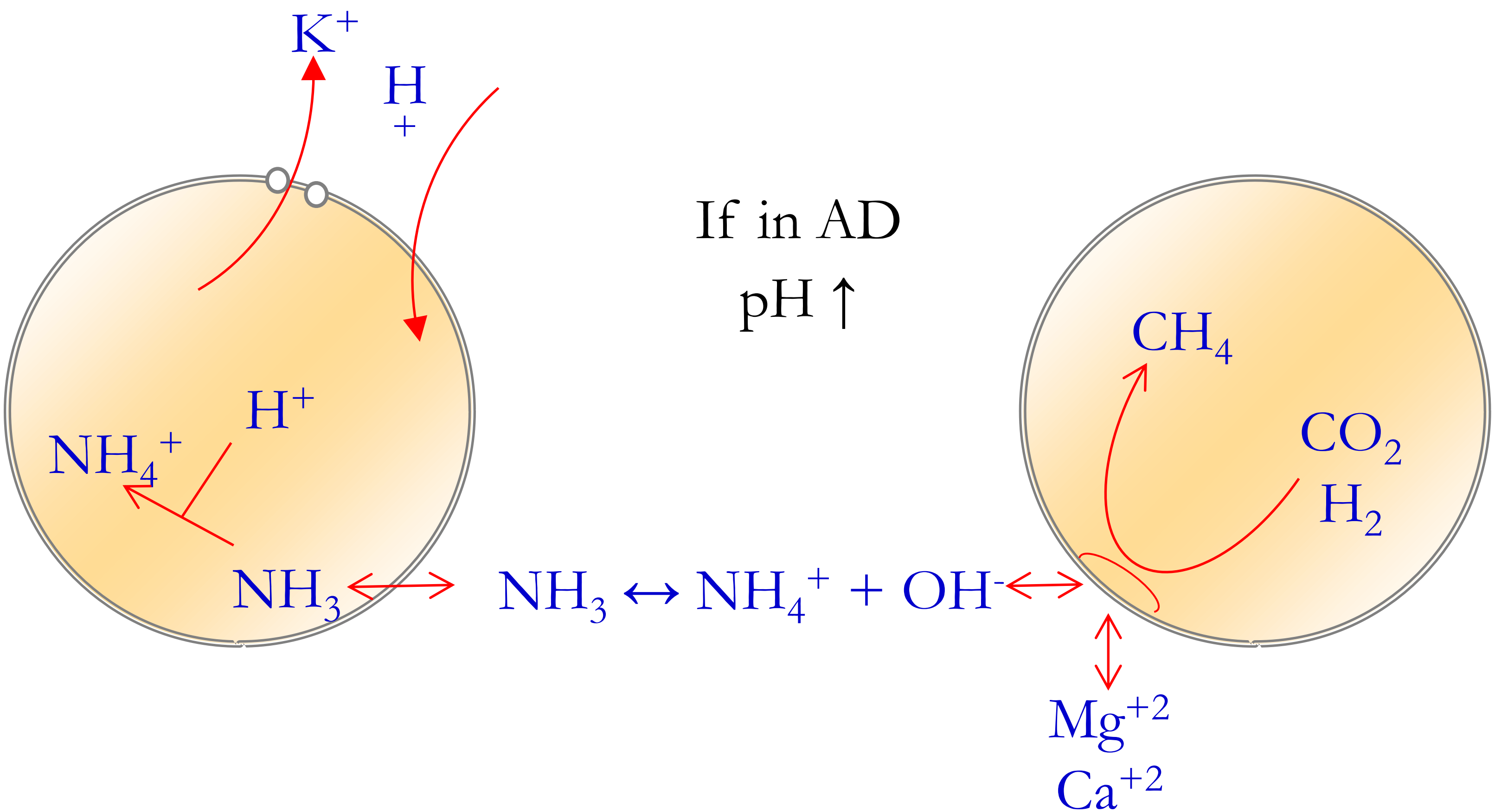
# Ammonia inhibition mechanism




Model illustrating two different interactions of ammonia with methanogens

# Effect of Ammonia on AD

**TABLE 17.4 Effects of Ammonical-nitrogen/Ammonia in an Anaerobic Digester**

| Ammonical-nitrogen ($NH_4^+$)/Dissolved Ammonia ($NH_3$), N | Effect |
|---|---|
| 50–200 mg/l | Beneficial |
| 200–1000 mg/l | No adverse effect |
| 1500–3000 mg/l | Inhibitory at pH > 7 |

Ref: Gerardi M.H. The microbiology of anaerobic digesters. Wiley Interscience. 2003

- Ammonia toxicity is "self-correcting".
  - Methanogens are inhibited by free ammonia
  - VFA concentration increases
  - Then pH of the digester drops.
  - The drop in pH converts FA to ammonium ions.

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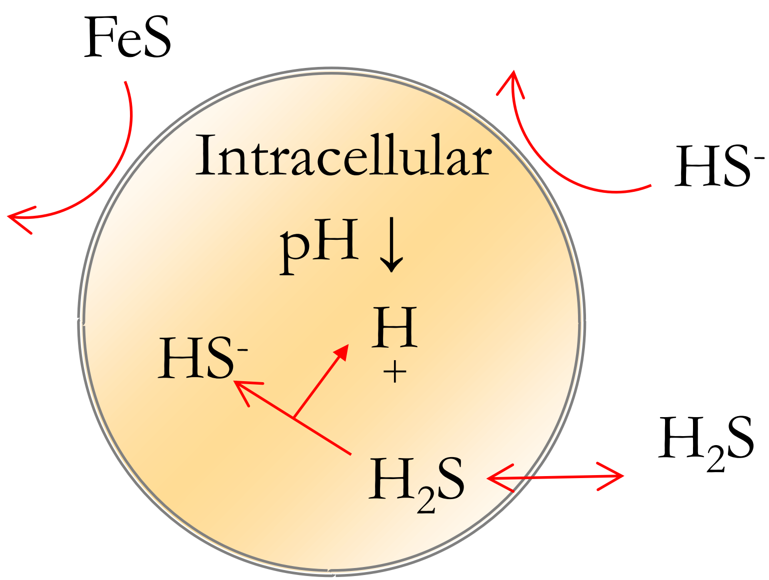
# Sulfide toxicity

- Bacterial cells need soluble sulfur as a growth nutrient and satisfy this need by using soluble sulfide ($HS^-$).
- However, excessive concentrations of sulfides or dissolved hydrogen sulfide ($H_2S$) gas cause toxicity.
- Methanogens are the microorganisms that are most susceptible to $H_2S$ toxicity in ADs.
- Hydrogenotrophic methanogens are more resistant to $H_2S$ than acetoclastic methanogens.

# Sulfide toxicity

- Soluble $H_2S$ toxicity occurs because sulfide inhibits the metabolic activity of anaerobic bacteria.
- Although the $H_2S$ inhibition mechanism is not completely understood, toxicity can occur at 200 mg/l at neutral pH.
- Because diffusion through a cell membrane is required to exert toxicity and non-ionized $H_2S$ diffuses more rapidly across a cell membrane than sulfide, $H_2S$ toxicity is pH dependent.

# Sulfide toxicity

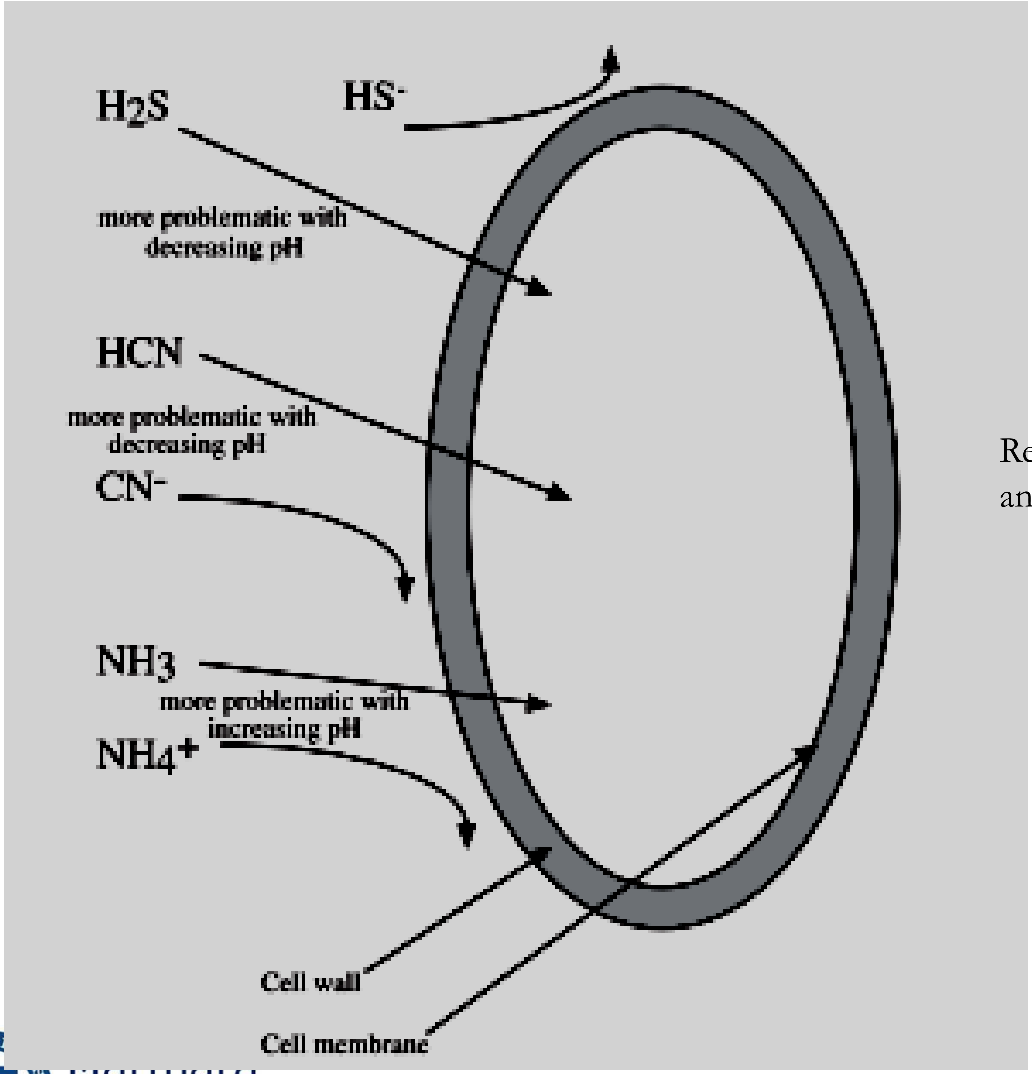



$H_2S \leftrightarrow HS^- + H^+$

If in AD

pH ↓

# pH dependent toxicity




Ref: Gerardi M.H. The microbiology of anaerobic digesters. Wiley Interscience. 2003

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# $H_2S$ formation in ADs

- $H_2S$ is formed in ADs from the reduction of sulfate and the degradation of organic compounds such as sulfur-containing amino acids and proteins.
- The sulfur in some of the amino acids is released during the hydrolysis.
- Sulfate is relatively non-inhibitory to methanogens.
- Sulfate is reduced to $H_2S$ by SRB.
- For each gr of COD degraded by SRB 1.5 gr of sulfate are reduced to $H_2S$.

# Control of $H_2S$ inhibition

- Free $H_2S$ gas can be stripped from digester sludge by the rapid production of $CO_2$, $H_2$ and $CH_4$.

Treatment measures include;

- Diluting the sulfides
- Increasing the pH to convert $H_2S$ to less toxic $HS^-$
- Separating and treating the sulfate/sulfide waste stream
- Precipitating the sulfide as a metal salt (FeS), and
- Scrubbing and recirculating digester biogas.

# VFA toxicity

- High concentrations of VFA are often associated with the effects of toxicity and inhibition.
- It is generally believed that VFA inhibition is due to their accumulation and a consequent reduction in pH value.
- However, several experiments have shown that the VFA are themselves toxic.

# VFA toxicity

- Depending on pH, VFA concentrations can be tolerated with a minimal degree of toxicity.
- However, at low pH values much more of the VFAs exists in the undissociated form which is much more toxic than ion form, due to its greater membrane permeability.
- In a well-operating digester running with lightly loaded feed, VFA concentration is typically less than 100 mg/l.

# VFAs in AD

VFAs generally present in AD process

| | |
|---|---|
| Formic acid | $HCOOH$ |
| Acetic acid | $CH_3COOH$ |
| Propionic acid | $CH_3\ CH_2COOH$ |
| Butyric acid | $CH_3\ CH_2\ CH_2COOH$ |
| Valeric acid | $CH_3\ CH_2\ CH_2\ CH_2COOH$ |
| Hexanoic acid | $CH_3\ CH_2\ CH_2\ CH_2\ CH_2COOH$ |
| Heptanoic acid | $CH_3\ CH_2\ CH_2\ CH_2\ CH_2\ CH_2COOH$ |
| Octanoic acid | $CH_3\ CH_2\ CH_2\ CH_2\ CH_2CH_2\ CH_2COOH$ |

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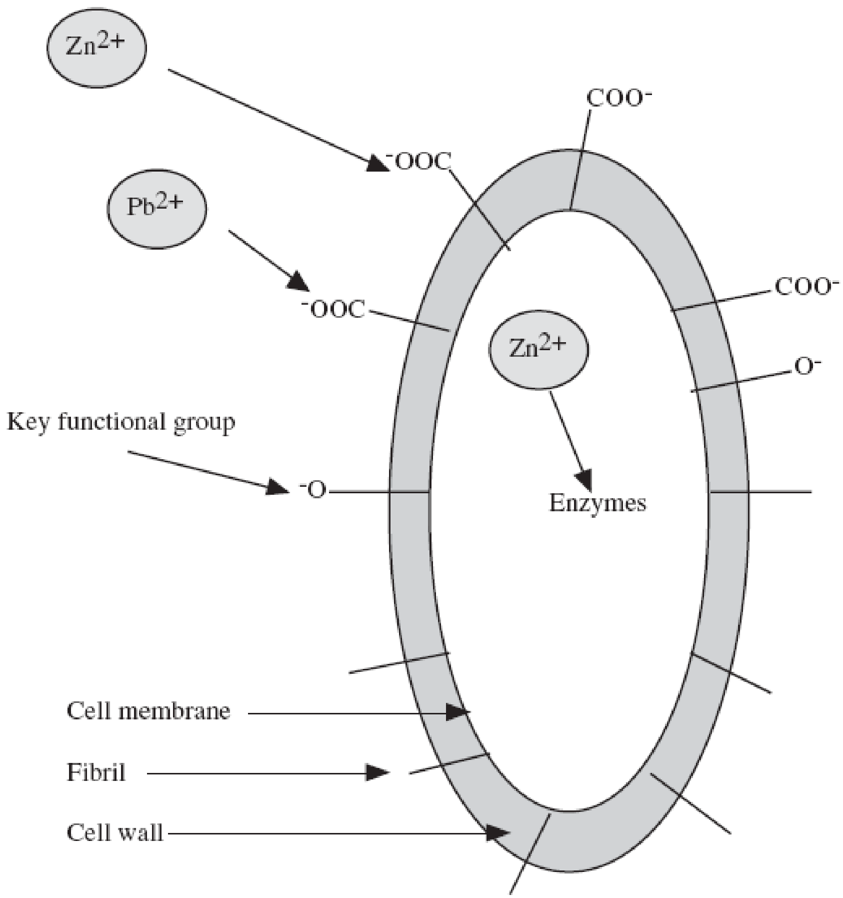
# Metal toxicity

- A trace level of many metal ions is required for the function of certain enzymes and coenzymes.
- However, excessive amounts may result in toxicity or inhibition.
- Heavy metal toxicity is believed to occur through the structural disruption of enzymes and protein molecules within the cell.

# Metal toxicity

- Numerous heavy metals such as cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) are found in wastewaters and sludges and are transferred to ADs.
- Copper, nickel, zinc, cadmium and mercury can be toxic to AD microflora at concentrations of <u>less than 1 mg/L</u>
- High concentrations of metals in sludges affect sludge disposal options and costs.

# Heavy Metals Toxicity




- Metals are adsorbed to the surface of negatively charged, bacterial fibrils that extend into bulk solution from cell membrane through cell wall.
- Fibrils are negatively charged by the ionization from key functional groups such as -COOH and hydroxyl -OH.
- Once adsorbed, metals are absorbed by bacterial cells.
- Inside the cells metals attack enzyme systems.

# Heavy Metals Toxicity

Heavy metal concentrations (mg/l) that cause a 50% reduction in biogas production rate

| | |
|---|---|
| Zinc | 163.0 |
| Cadmium | 180.0 |
| Copper | 170.0 |
| Nickel | 0.6 |
| Lead | 2.0 |

# Salt Toxicity

- Alkali and alkaline earth metals (sodium, potassium, magnesium, and calcium) are stimulatory to anaerobic bacteria unless present at excessive concentrations.
- The toxicity of salts of these metals is associated with the cation rather than anion.
- Aacclimatization of digester with cations can often increase the toxicity threshold.

# Salt Toxicity

•Inhibitory concentrations of alkali and alkaline–earth cations

| *Cation* | *Concentrations in mg/l* | |
|---|---|---|
| | *Moderately inhibitory* | *Strongly inhibitory* |
| Sodium | 3500–5500 | 8000 |
| Potassium | 2500–4500 | 12 000 |
| Calcium | 2500–4500 | 8000 |
| Magnesium | 1000–1500 | 3000 |

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# Cyanide toxicity

- Cyanide (-CN) and cyanide-containing compounds are commonly found in wastewaters from metal cleaning and electroplating industries.
- In metal finishing industry they are used in plating baths.
- Cyanide & cyano-compounds are toxic to methanogens.
- Toxicity occurs at cyanide concentrations >100 mg/l.

# Cyanide toxicity

- Cyanide prevents methane production from acetate, but it may not prevent methane production from $H_2 + CO_2$.
- Cyanide toxicity is reversible.
- The reversibility of toxicity is dependent on;
  - Concentration of cyanide and its time in the digester
  - Amounts of solids (bacteria) in digester
  - Solids retention time (SRT), and
  - Temperature.

# Toxicity of Anthropogenic & Recalcitrant Compounds

- Chlorinated Hydrocarbons
- Benzene Ring Compounds:
  - Benzene
  - Pentachlorophenol
  - Phenol and phenolic compounds (chlorophenols, nitrophenols and tannins)
  - Toluene.
- Formaldehyde ($H_2CO$)

# Recalcitrant Compounds

- Difficult to degrade or recalcitrant compounds in anaerobic digesters may cause toxicity to methanogens.
- Examples of these compounds include aliphatic hydrocarbons and some chlorinated compounds such as lignin, humic substances, and chlorinated biphenyls.
- The recalcitrant compounds become even more difficult to degrade when they contain alkyl groups, halogens, nitro groups, and sulfo groups.