Chapter 5

Toxic Substances in Anaerobic Digestion

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.
- Organic compounds such as oils are removed in scum and chloroform in sludge.
- Industrial wastewaters often contain wastes that are toxic to anaerobic digesters.

Marmara Üniversitesi

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 in AD may be acute or chronic.

- Acute toxicity results from the rapid exposure of an unacclimated population of bacteria to a relatively high concentration of a toxic waste.
- Chronic toxicity results from the gradual and relatively long exposure of an unacclimated population of bacteria to a toxic waste.

Marmara Üniversitesi

Chronic toxicity

Under chronic toxicity anaerobic bacteria & methanogens may acclimate 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.

Marmara Üniversitesi

Toxic substances in AD

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

Marmara Üniversitesi

Toxic substances in AD

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

Marmara Üniversitesi

Measures to control toxicity in AD

- Remove toxic substances from the feed
- Dilute the feed to bring levels below toxic threshold value
- Add chemicals to form a non-toxic complex or insoluble precipitate
- Add an antagonistic substance.

Marmara Üniversitesi

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.

Marmara Üniversitesi

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.

Marmara Üniversitesi

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	$CH_3\ CH_2\ CH_2\ CH_2\ CH_2CH_2\ CH_2COOH$

Marmara Üniversitesi

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.

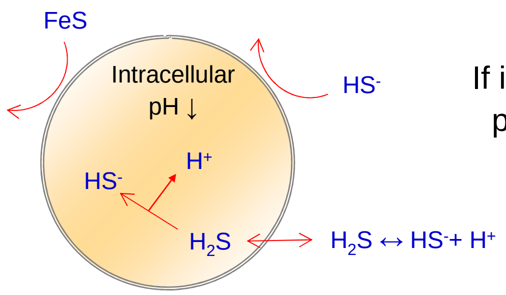
Marmara Üniversitesi

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.

Marmara Üniversitesi

Sulfide Inhibition Mechanism



If in AD
pH ↓

Hydrogen sulfide (H_2S) inhibition mechanism

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.

To control 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.

Marmara Üniversitesi

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.
- 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 toxic especially to methanogens.

Marmara Üniversitesi

Forms of Ammonia

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

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 N, mg/l

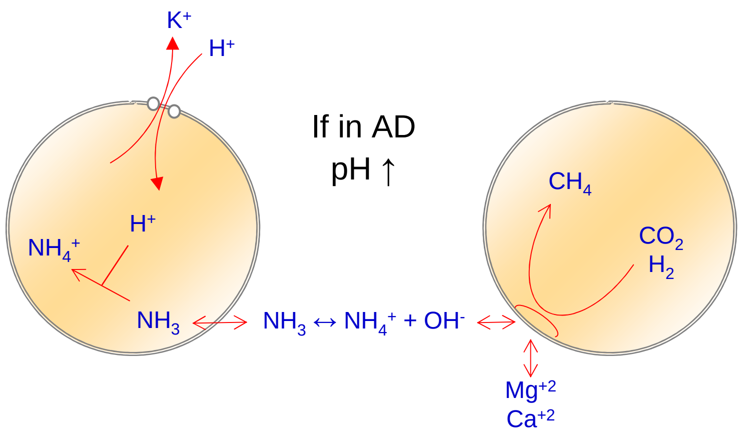
FA : Free NH_3-N, mg/l

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

T : Temperature, °C

Marmara Üniversitesi

Ammonia Inhibition Mechanism



Model illustrating two different interactions of ammonia with methanogens

Marmara Üniversitesi

Effect of Ammonia on AD

Total Ammonia-N	***Effect***
50–200mg/l	Beneficial
200–1000mg/l	No adverse effect
1500–3000mg/l	Inhibitory at pH > 7

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.

Marmara Üniversitesi

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.

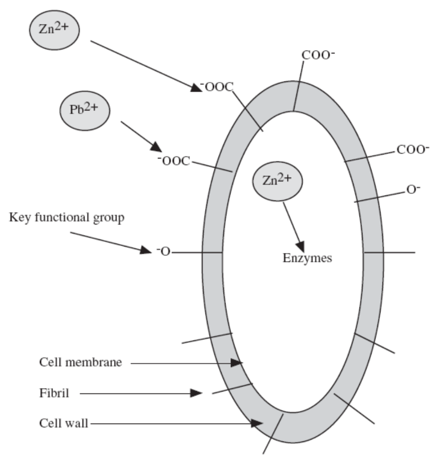
Marmara Üniversitesi

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.
- High concentrations of metals in sludges affect sludge disposal options and costs.

Marmara Üniversitesi

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.

Marmara Üniversitesi

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

Alkali & Alkaline-earth Metals 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.
- Acclimatization of digester with cations can often increase the toxicity threshold.

Alkali & Alkaline-earth Metals 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

Marmara Üniversitesi

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.

Marmara Üniversitesi

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.

Marmara Üniversitesi

Toxicity of Anthropogenic & Recalcitrant Compounds

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

Marmara Üniversitesi

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.

Marmara Üniversitesi