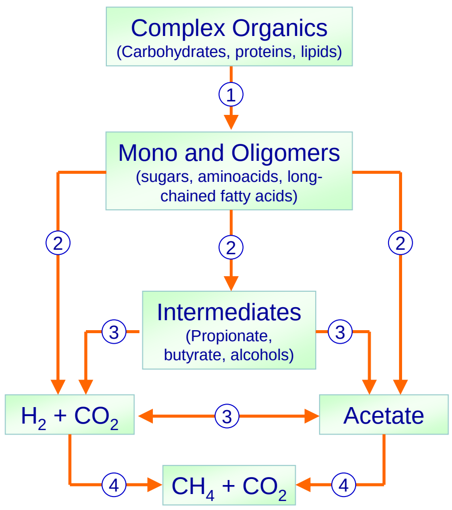
Chapter 2

Biochemistry of Anaerobic Digestion

Marmara Üniversitesi

Anaerobic Digestion



1. Hydrolysis
2. Fermentation
3. Acetogenesis
4. Methanogenesis

Marmara Üniversitesi

Hydrolysis

- Solubilization of insoluble particles and biological decomposition of organic polymers to monomers or dimers, which can pass through cell membrane.
- Usually carried out by extracellular enzymes.
- It is not necessarily an enzymatic process catalyzed by biologically produced enzymes but could take place due to physico-chemical reactions as well.
- Hydrolysis of a complex, insoluble substrate depends on different parameters such as; (i) Particle size (ii) pH (iii) production of enzymes and (iv) diffusion and adsorption of enzymes to particles

Hydrolysis

Substrate	Hydrolysis rate, d^{-1}	Ref.
Carbohydrates	0.025-0.200	1
Cellulose	0.040-0.130	2
Proteins	0.015-0.075	1
Lipids	0.005-0.010	1

[1] Christ O,Wilderer PA,Angerhofer R,Faulstich M (2000) Water Sci Technol 41:61
[2] Gujer W, Zehnder AJB (1983) Water Sci Technol 15:127

Marmara Üniversitesi

Fermentation (Acidogenesis)

- Dissolved organic matter is biodegraded mainly to volatile fatty acids (VFAs) and alcohols by a heterogeneous microbial population.
- Dominant species in anaerobic digesters are bacteria while small populations of protozoa, fungi and yeasts have also been reported.
- It is mainly the obligatory and facultative anaerobic bacteria that carry out the fermentation.
- Most important factors that influence the fermentation are; (i) interspecies hydrogen transfer (ii) pH (iii) hydraulic retention time (iv) previous acclimation of the anaerobic culture

Marmara Üniversitesi

Acetogenesis

- Oxidation of fermentation products into a substrate (acetate, H_2 and CO_2) appropriate for methanogens.
- *Homoacetogenesis*: Production of acetate as a sole end product from CO_2 and H_2.

  Thermodynamically, it is less favorable than methano-genesis and sulfate reduction.
- *Synthrophic Acetogenesis*: Anaerobic oxidation of propionate and butyrate to acetate and H_2.

  Propionate and butyrate oxiditation are inhibited by even low H_2 partial pressures, therefore can occur only if H_2 is consuming by methanogens, SRB and homoacetogens.

Marmara Üniversitesi

Acetogenesis

Syntrophic Acetogenesis

- Propionate⁻ + 3 H_2O → Acetate⁻ + HCO_3^- + H^+ + 3 H_2
  ΔG_0* = +76.1 kJ/mol substrate
- Butyrate⁻ + 2 H_2O → 2 Acetate⁻ + H^+ + 2 H_2 Consumed
  ΔG_0* = +48.3 kJ/mol

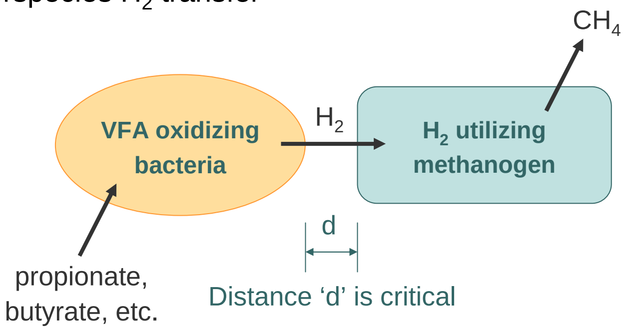
Homoacetogenesis

- 4 H_2 + 2 HCO_3^- + H^+ → Acetate⁻ + 4 H_2O
  ΔG_0* = -104.6 kJ/mol

* Thauer RK, Jungermann K, Decker K (1977) Bacteriol Rev 41:100

Syntrophic Acetogenesis

Interspecies H_2 transfer



The lower the H_2 concentration the better are the thermodynamics of the VFA degradation

Methanogenesis

- A limited number of organic compounds are used as carbon and energy sources in methanogenesis.
- They are; CO_2, CO, formic and acetic acid, methanol, methylamines and dimethyl sulfide.
- Almost 65-70% of CH_4 produced in anaerobic digesters comes from acetate.
- Methanogenesis from CO_2 and H_2 has a significant role as well by keeping a low hydrogen pressure and thus supporting the anaerobic oxidation VFAs to acetate & H_2.
- Methanogenesis is extremely sensitive to temperature, loading rate and pH fluctuations and inhibited by a number of organic & inorganic compounds.

Marmara Üniversitesi

Methanogenesis

- $4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$

  $\Delta G_0 = -135.5$ kJ/mol substrate
- $Acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$

  $\Delta G_0 = -32.3$ kJ/mol
- $Methanol \rightarrow ¾\ CH_4 + ¼\ HCO_3^- + ¼\ H^+ + ¼\ H_2O$

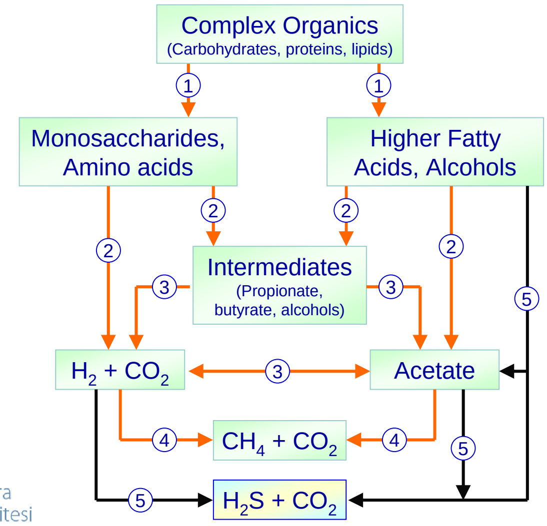
  $\Delta G_0 = -79.9$ kJ/mol
- $Formate^- + H^+ \rightarrow ¼\ CH_4 + ¾\ CO_2 + ½\ H_2O$

  $\Delta G_0 = -36.1$ kJ/mol

* Thauer RK, Jungermann K, Decker K (1977) Bacteriol Rev 41:100

Marmara Üniversitesi

AD with Sulfate Reduction



1. Hydrolysis
2. Fermentation
3. Acetogenesis
4. Methanogenesis
5. Sulfate reduction

Marmara Üniversitesi

Sulfate Reduction

- Sulfate (SO_4^{2-}) or sulfite (SO_3^{2-}) can be used by SRB as acceptor of electrons released during the oxidation of organic materials under anaerobic conditions.
- The end product is hydrogen sulfide (H_2S).
- VFAs, several aromatic acids, H_2, methanol, ethanol, glycerol, sugars, amino acids and some phenol compounds are the substrates used in sulfate reduction.
- There is a competition for the substrate available to be used in sulfate reduction instead of fermentation (sugars), acetogenesis (VFAs) and methanogenesis (acetate, H_2).
- SO_4^{2-}/COD ratio is the critical parameter.

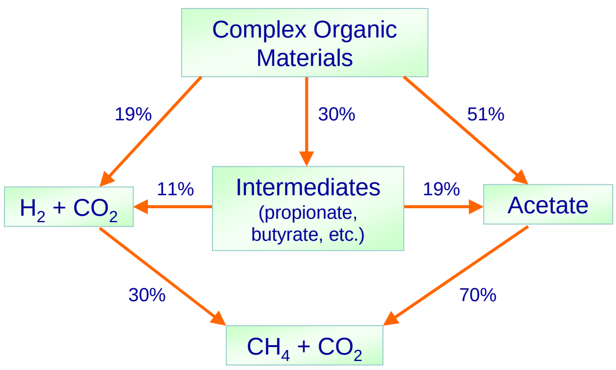
Marmara Üniversitesi

Sulfate Reduction

- $4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$
  ΔG_0 = -151.9 kJ/mol substrate
- $Acetate^- + SO_4^{2-} \rightarrow 2\ HCO_3^- + HS^-$
  ΔG_0 = -47.6 kJ/mol
- $Propionate^- + ¾\ SO_4^{2-} \rightarrow Acetate^- + HCO_3^- + ¾\ HS^- + ¼\ H^+$
  ΔG_0 = -37.7 kJ/mol
- $Butyrate^- + ½\ SO_4^{2-} \rightarrow 2\ Acetate^- + ½\ HS^- + ½\ H^+$
  ΔG_0 = -27.8 kJ/mol
- $Lactate^- + ½\ SO_4^{2-} \rightarrow Acetate^- + HCO_3^- + ½\ HS^- + ½\ H^+$
  ΔG_0 = -80.0 kJ/mol
- $Ethanol + ½\ SO_4^{2-} \rightarrow Acetate^- + ½\ HS^- + ½\ H^+ + H_2O$
  ΔG_0 = -66.4 kJ/mol

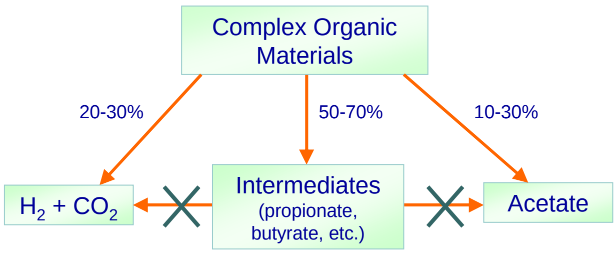
* Thauer RK, Jungermann K, Decker K (1977) Bacteriol Rev 41:100

Anaerobic Digestion



Carbon flow in anaerobic environments with active methanogens

Anaerobic Digestion

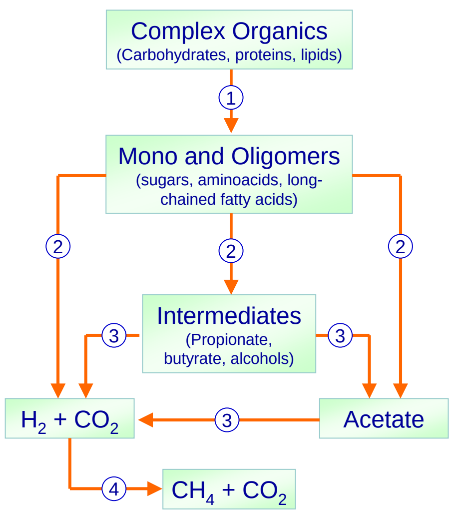


X: Inhibited because of high H_2 partial pressure

Carbon flow in anaerobic environments without active methanogens

Marmara Üniversitesi

Syntrophic Acetate Conversion



1. Hydrolysis
2. Fermentation
3. Acetogenesis
4. Methanogenesis

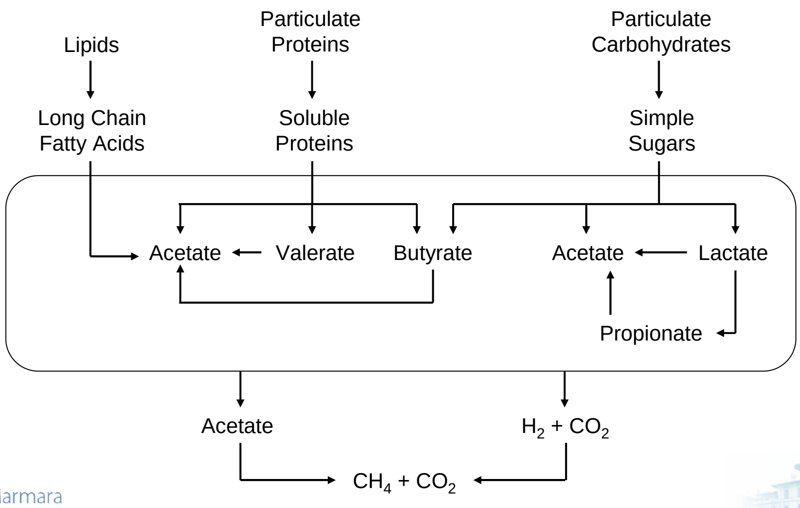
Marmara Üniversitesi

Syntrophic Acetate Conversion

- When acetate-utilizing methanogens are inhibited by high concentrations of ammonia or sulfite, other groups of microorganisms replace them to obtain energy from oxidation of acetate to H_2 & CO_2.
- Due to thermodynamic constrains this reaction proceeds much better at temperatures higher than 60℃ (upper limit of thermophilic acetate-utilizing methanogens) and is the way of acetate transformation.
- Both syntrophic acetate oxidation and methanogenesis from acetate can be simultaneously occur in an AD.

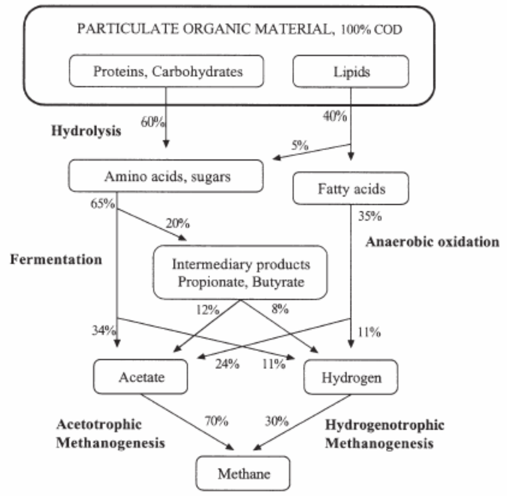
Marmara Üniversitesi

Biochemical Pathways



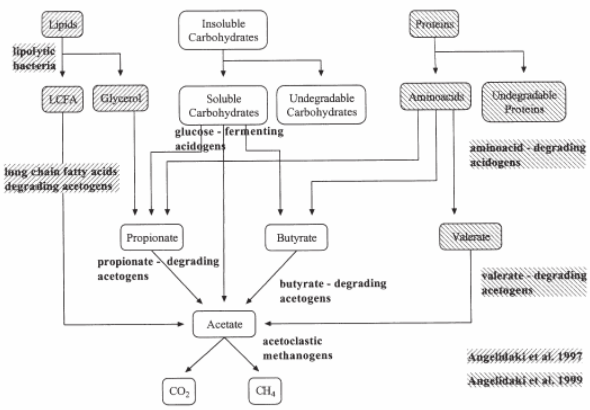
Marmara Üniversitesi

AD of Sewage Sludge



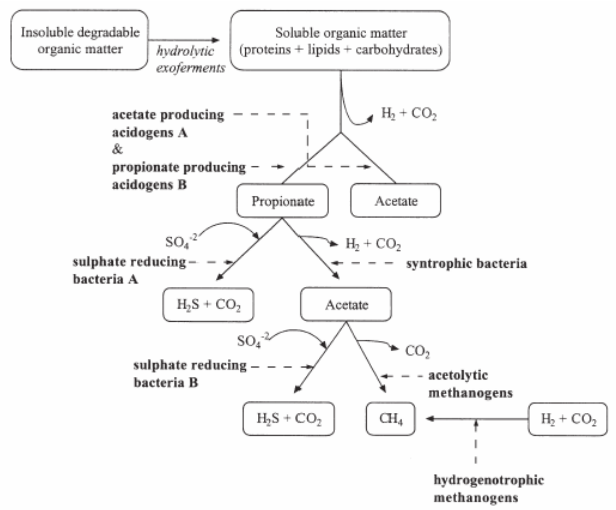
Marmara Üniversitesi

AD of Lipids & Proteins



Marmara Üniversitesi

AD with Sulfate Reduction



Marmara Üniversitesi