ENVE 424
Anaerobic Treatment

Lecture 2
Biochemistry of Anaerobic Treatment

2012 – 2013 Fall

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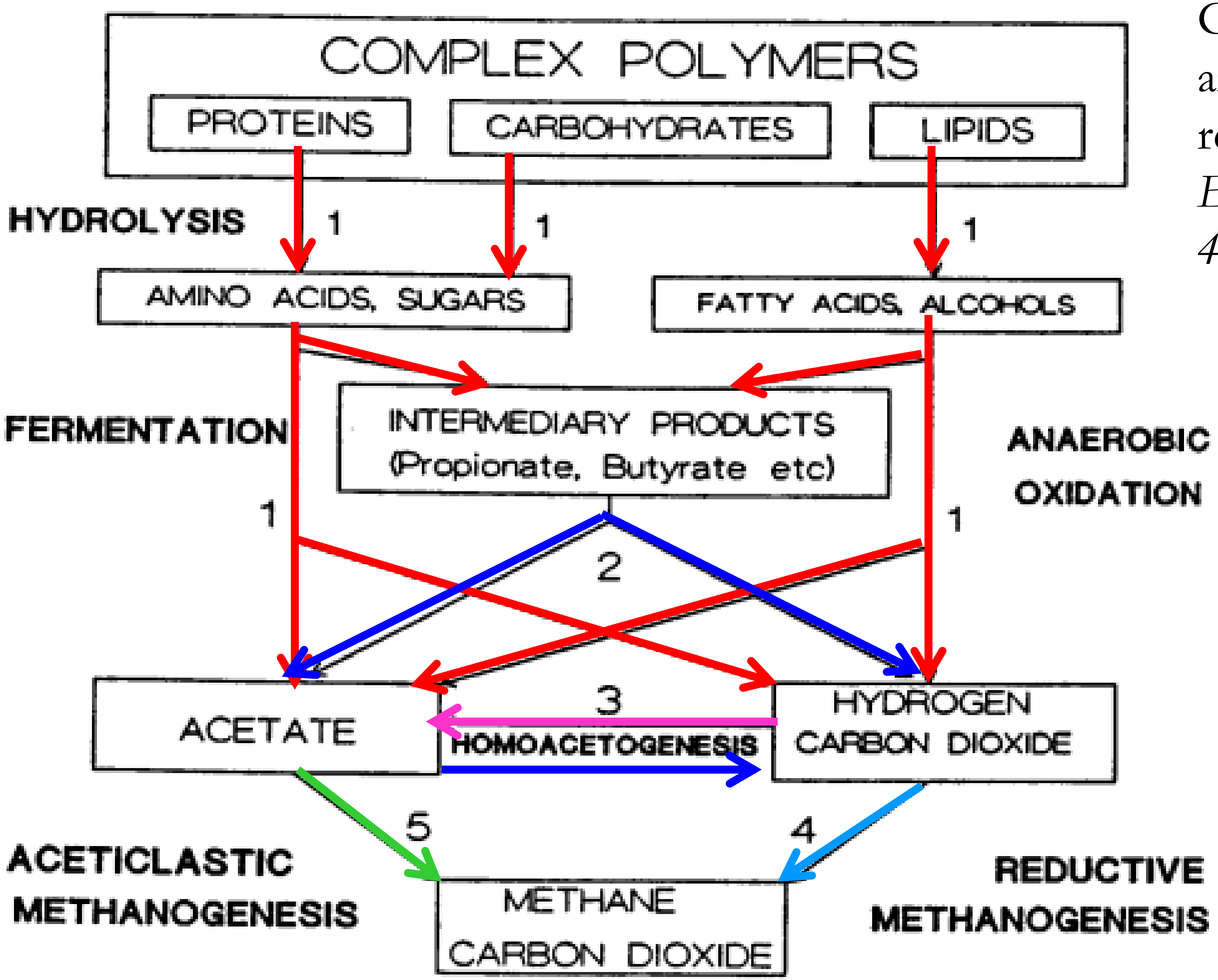
- Lecture notes are prepared by Prof. Dr. B. Callı and Assist. Prof. Dr. A. E. Tugtas

Anaerobic Fermentation/Digestion

- Anaerobic treatment processes have been applied for centuries
    - Higher loading rates,
    - low sludge production,
    - sustainable energy-producing technology,
    - nutrient conservation and
    - pathogen control (thermophilic AD)

Anaerobic Digestion



Ref: Pavlostathis, S.G.; Giraldo-Gomez, E. (1991) Kinetics of anaerobic treatment - A critical review. *Critical Reviews in Environmental Control, 21 (5-6): 411-490.*

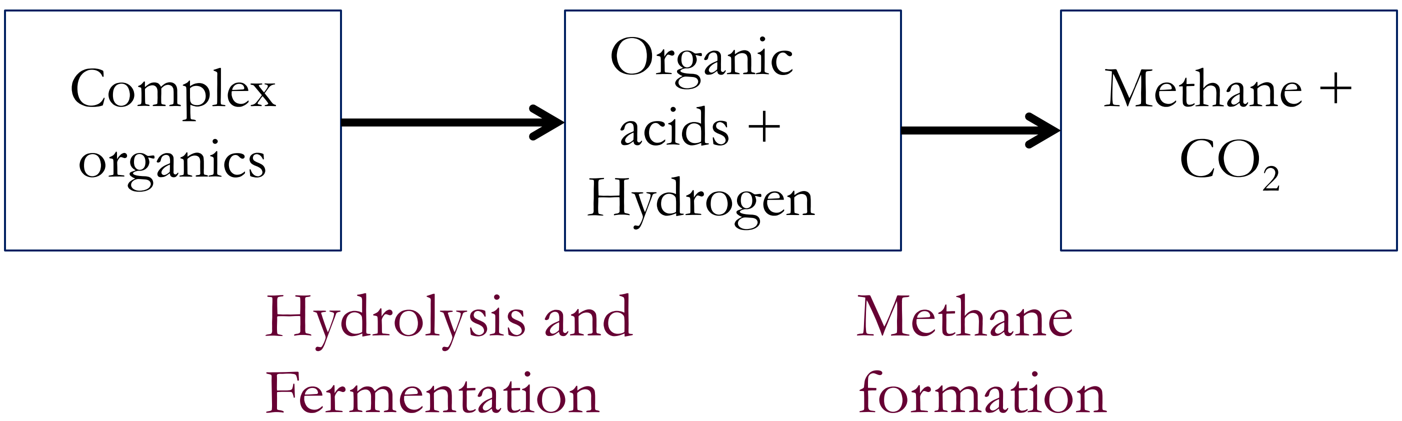
<u>Numbers indicate bacterial groups</u>

1. **Fermentative bacteria**
2. **Hydrogen producing acetogenic bacteria**
3. **Hydrogen consuming acetogenic bacteria**
4. **CO2 reducing methanogens**
5. **Aceticlastic methanogens**

Anaerobic Digestion/Fermentation

- Mixed microbial community is involved in anaerobic digestion
- Thermodynamics and kinetics are crucial to the mixed microbial community
- Anaerobic digestion process can be broken into two basic principles
  - Hydrolysis and fermentation of complex organic matter into simple organic acids and hydrogen
  - Conversion of organic acids and hydrogen into methane

Anaerobic Digestion



Organic Acids

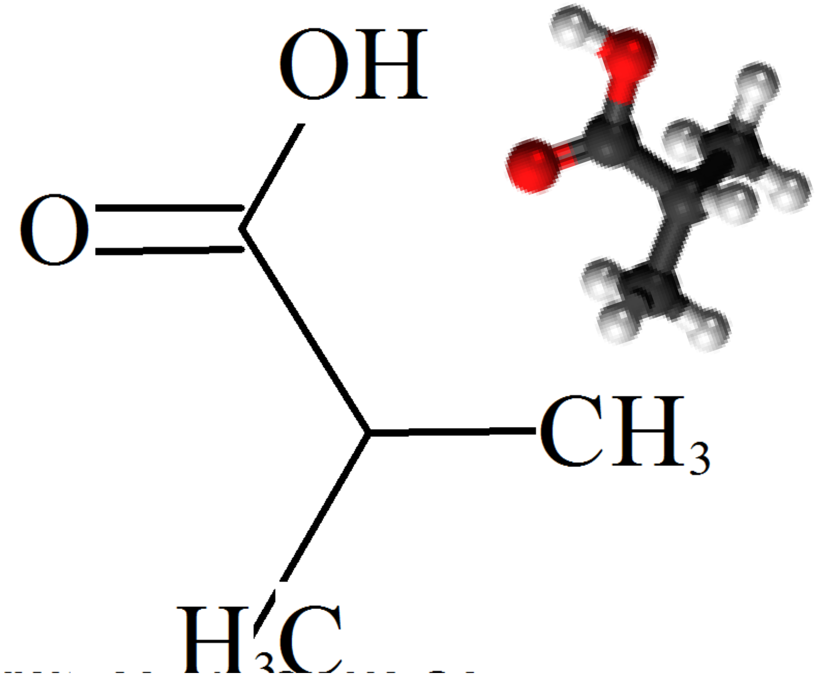
Volatile Acids

- Formic Acid
- **Acetic Acid**
- **Propionic Acid**
- **N-Butyric aAcid**
- **IsoButyric Acid**
- N-Valeric Acid
- Isovaleric Acid
- Caproic Acid
- Heptanoic Acid
- Octanoic Acid

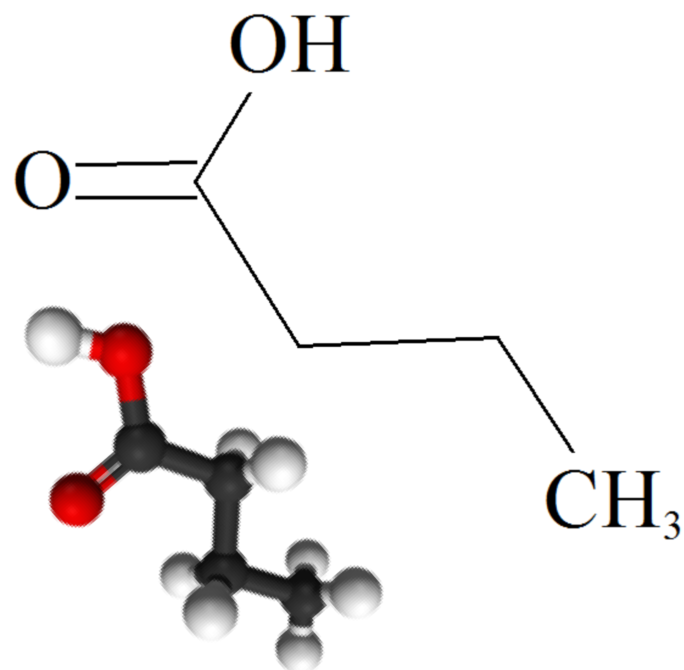
Non-volatile Acids

- Lactic Acid
- Pyruvic Acid
- Succinic Acid

Isomers



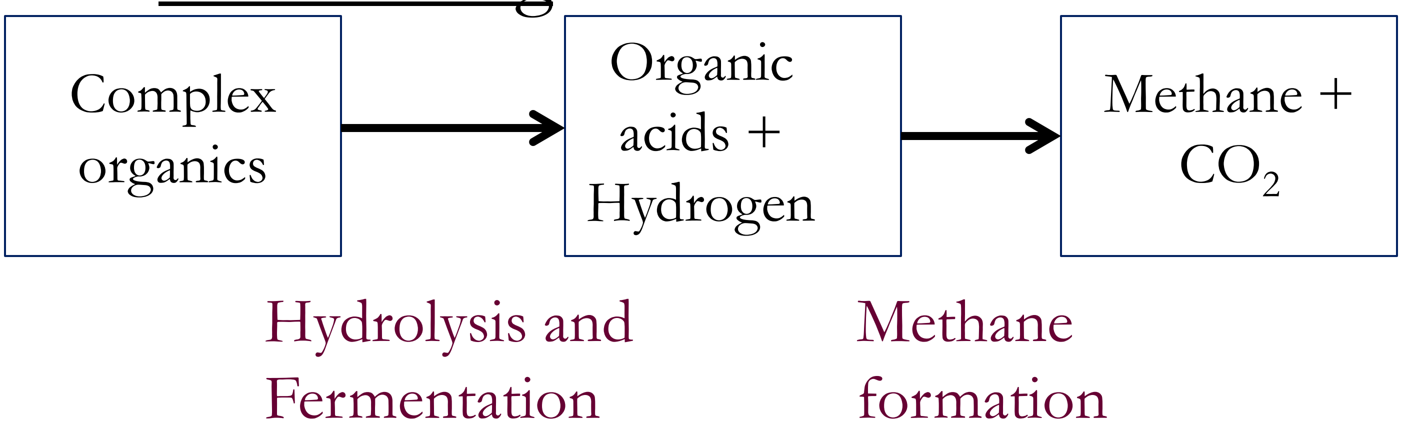
N-Butyric Acid
$CH_3CH_2CH_2COOH$
$C_4H_8O_2$



Isobutyric Acid
$CH_3CH_2CH_2COOH$
$C_4H_8O_2$

Anaerobic Digestion

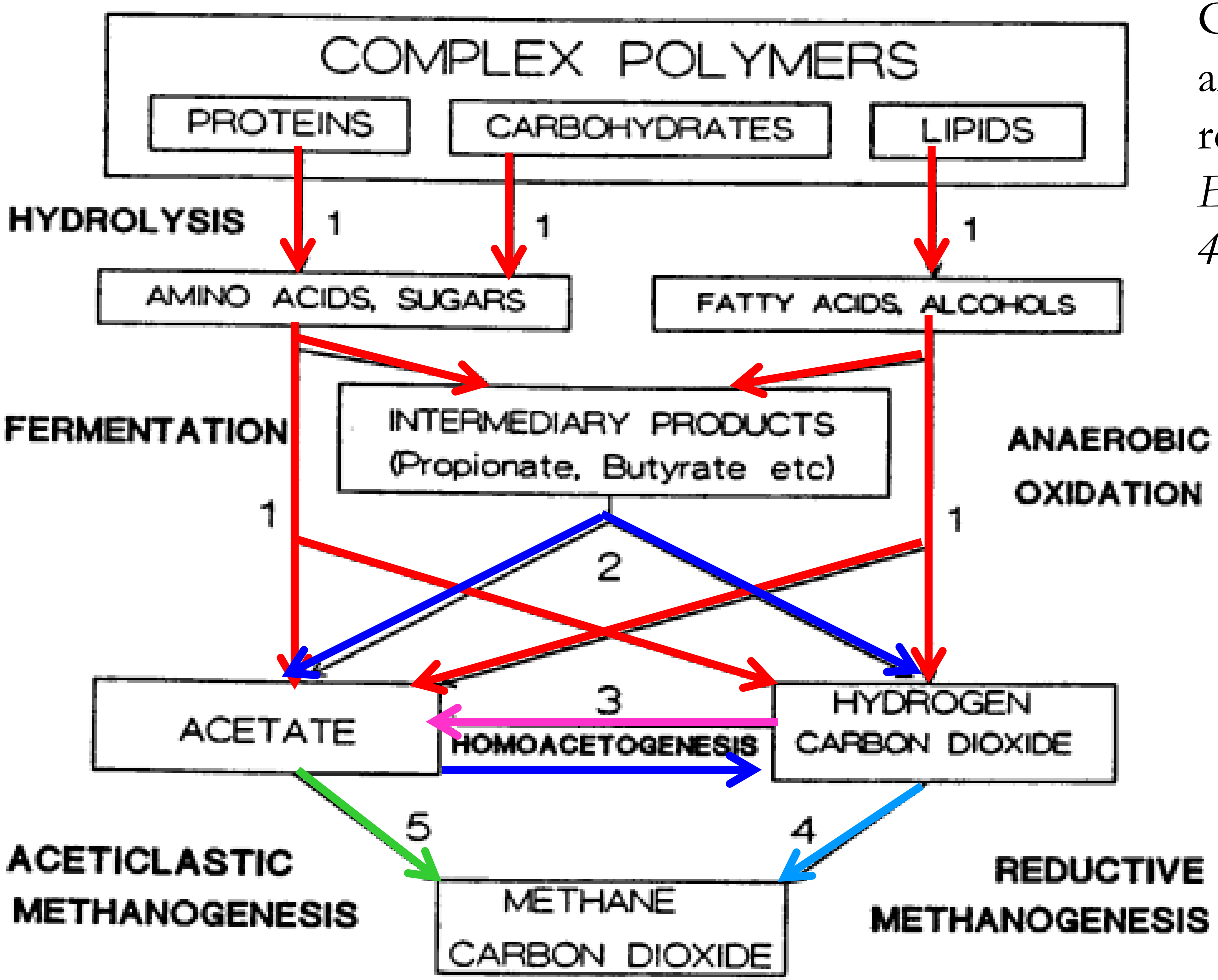
- Microorganisms involved hydrolysis and fermentation grow more rapidly – fermentation reactions yield more energy compared to that of methane formation
- Methanogens are more slowly growing and tend to be rate limiting



Anaerobic Digestion

- Methanogenesis is rate limiting for most of the organic matter
- Hydrolysis may be rate limitting for lignocellulosic materials such as grasses, agricultural crop residues, newspapers etc.
- For a succesful start-up and operation of an anaerobic system → proper balance needs to be maintained between hydrolytic and fermentative organisms

Anaerobic Digestion



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Hydrolysis

- Hydrolysis is the first step for most fermentation processes
- Particulate matter cannot pass through the cell membrane
- Particulate matter is converted to soluble compounds via <u>extracellular enzymes</u>
- Soluble compounds are small enough to pass through the cell membrane

Hydrolysis

- Soluble compounds are hydrolysed further to simple monomers that are used by the bacteria to perform fermentation
- Hydrolysis is a <u>first order enzymatic process</u>
- 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

Hydrolysis

Parameter	Description	Feed	Dead Biomass
k_{dis} (d^{-1})	Disintegration rate	-	2
k_{hyd_CH} (d^{-1})	Hydrolysis rate for carbohydrates	2	0.15
k_{hyd_PR} (d^{-1})	Hydrolysis rate for proteins	2	0.5
k_{hyd_Li} (d^{-1})	Hydrolysis rate for lipids	2	0.15

•Batstone DJ, Keller J, Angelidaki RI, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA. 2002. *Anaerobic Digestion Model No. 1. (ADM1)*, IWA Task Group for Mathematical Modelling of Anaerobic Wastewater Processes, Scientific and Technical Report No. 13, IWA Publishing, London, UK.

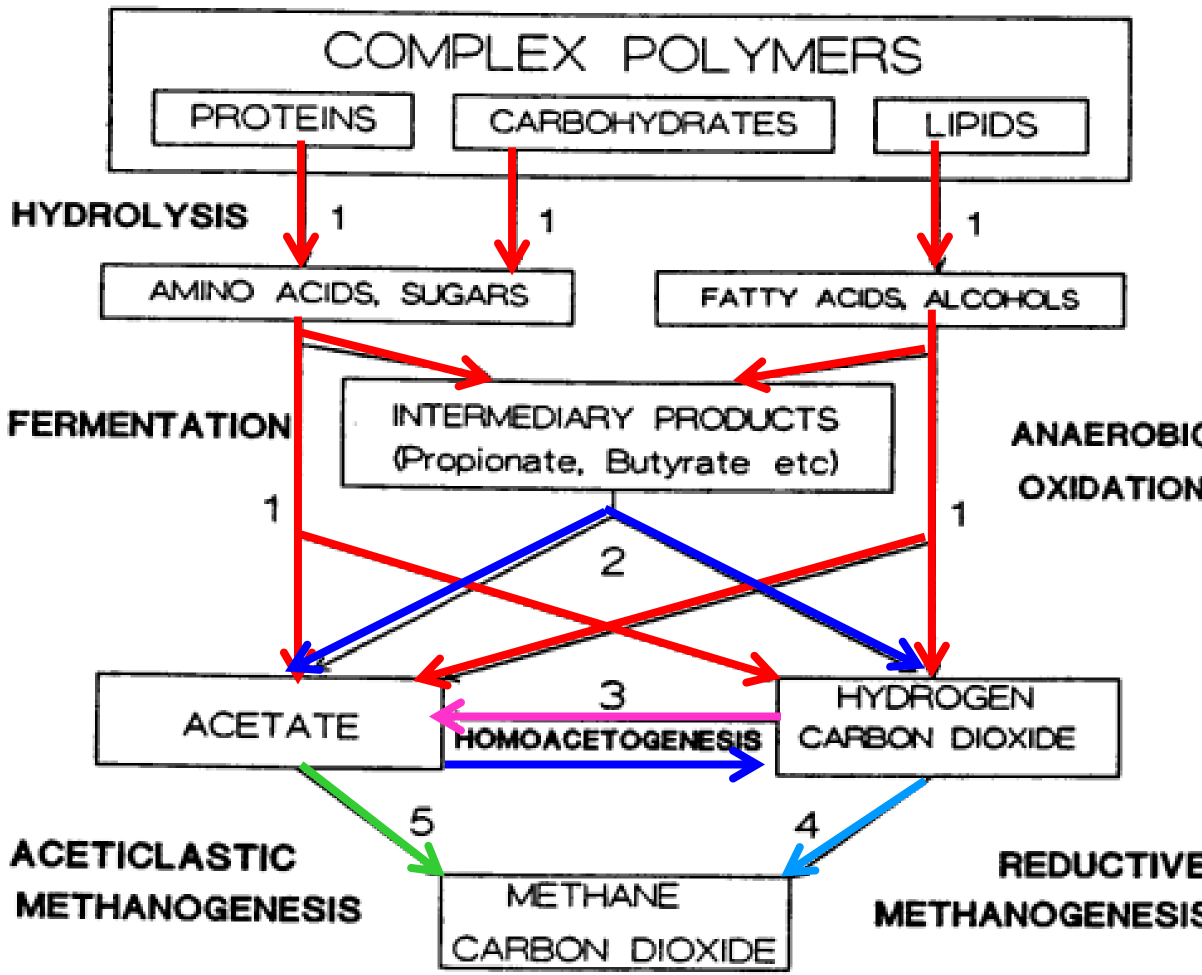
•Tugtas AE, Tezel U, Pavlostathis SG. 2006. An extension of the Anaerobic Digestion Model No.1 to include the effect of nitrate reduction processes. Water Sci Technol 54:41-49.

Fermentation (Acidogenesis)

- Dissolved organic matter is biodegraded mainly to volatile fatty acids (VFAs) and alcohols by a heterogeneous microbial population.
- Organic matter serves as electron donors and acceptors
- The principal products of fermentation are acetate, hydrogen, CO_2, propionate, and butyrate

Fermentation (Acidogenesis)

The free energy change associated with the conversion of **propionate and butyrate** to **acetate and hydrogen** requires hydrogen to be at low concentrations **(H_2<10^{-4} atm)**



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Fermentation (Acidogenesis)

- 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

Acetogenesis

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

  Thermodynamically, it is less favorable than methanogenesis and sulfate reduction.

Acetogenesis

- *Synthrophic Acetogenesis*: Anaerobic oxidation of propionate and butyrate to acetate and H_2.

  Propionate and butyrate oxidation are inhibited by H_2 partial pressures above **10^{-4} atm. The process can only occur** if H_2 is consumed by methanogens, SRB and/or homoacetogens.

Acetogenesis

Syntrophic Acetogenesis

- $Propionate^{-} + 3\ H_2O \rightarrow Acetate^{-} + HCO_3^{-} + H^{+} + 3\ H_2$

  $\Delta G_0^* = +76.1$ kJ/mol substrate

- $Butyrate^{-} + 2\ H_2O \rightarrow 2\ Acetate^{-} + H^{+} + 2\ H_2$

  $\Delta G_0^* = +48.3$ kJ/mol

•Consumed

Homoacetogenesis

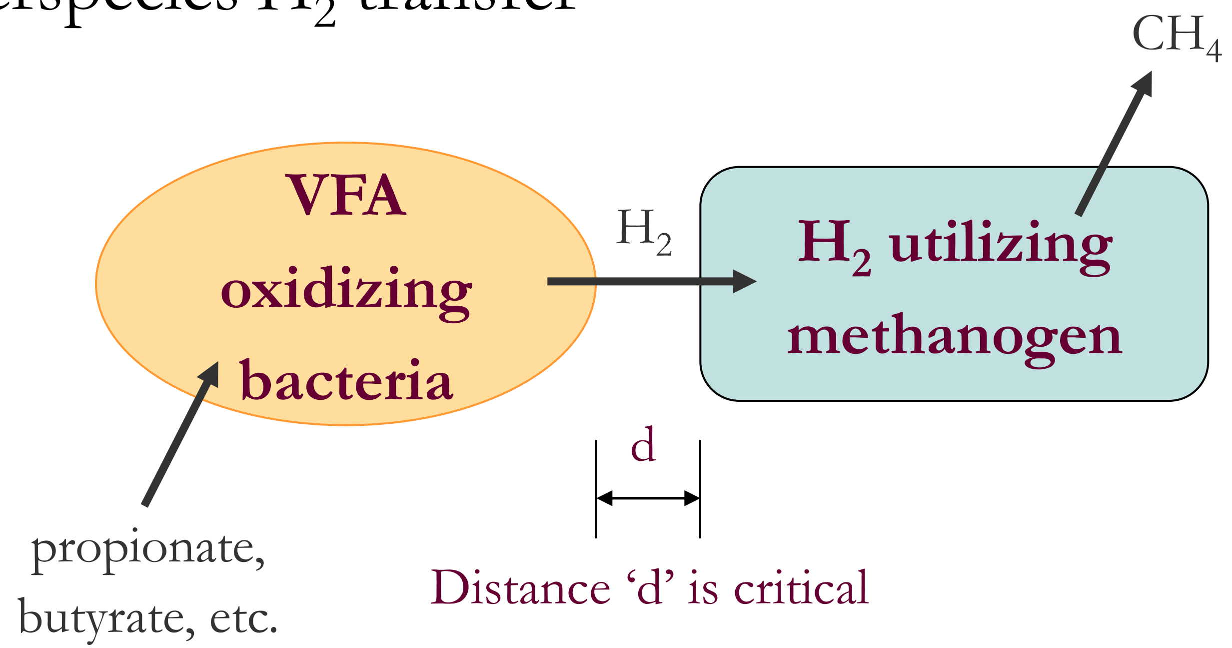
- $4\ H_2 + 2\ HCO_3^{-} + H^{+} \rightarrow Acetate^{-} + 4\ H_2O$

  $\Delta G_0^* = -104.6$ 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

- 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.

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

- $Methanol \rightarrow \frac{3}{4}\ CH_4 + \frac{1}{4}\ HCO_3^- + \frac{1}{4}\ H^+ + \frac{1}{4}\ H_2O$

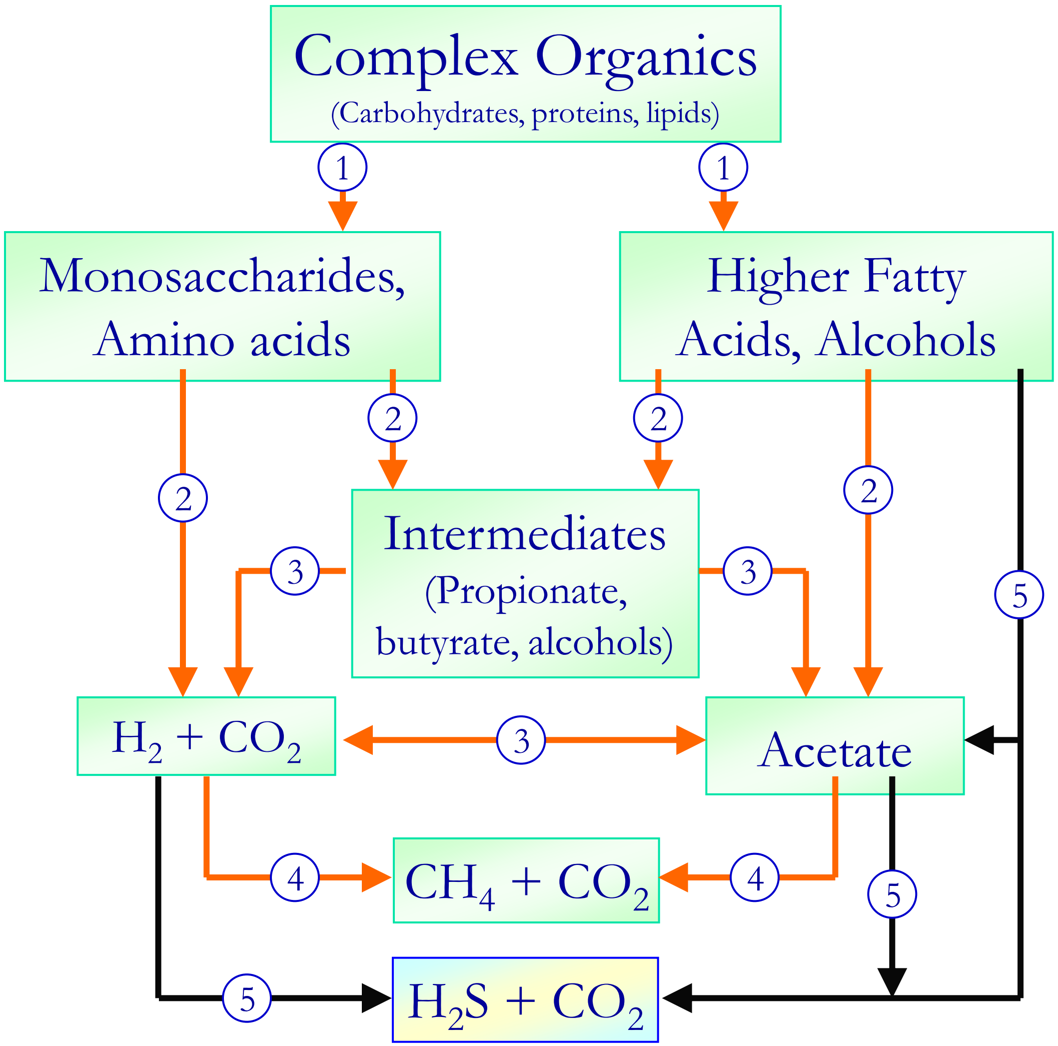
  $\Delta G_0 = -79.9$ kJ/mol

- $Formate^- + H^+ \rightarrow \frac{1}{4}\ CH_4 + \frac{3}{4}\ CO_2 + \frac{1}{2}\ H_2O$

  $\Delta G_0 = -36.1$ kJ/mol

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

AD with Sulfate Reduction



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

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.

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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

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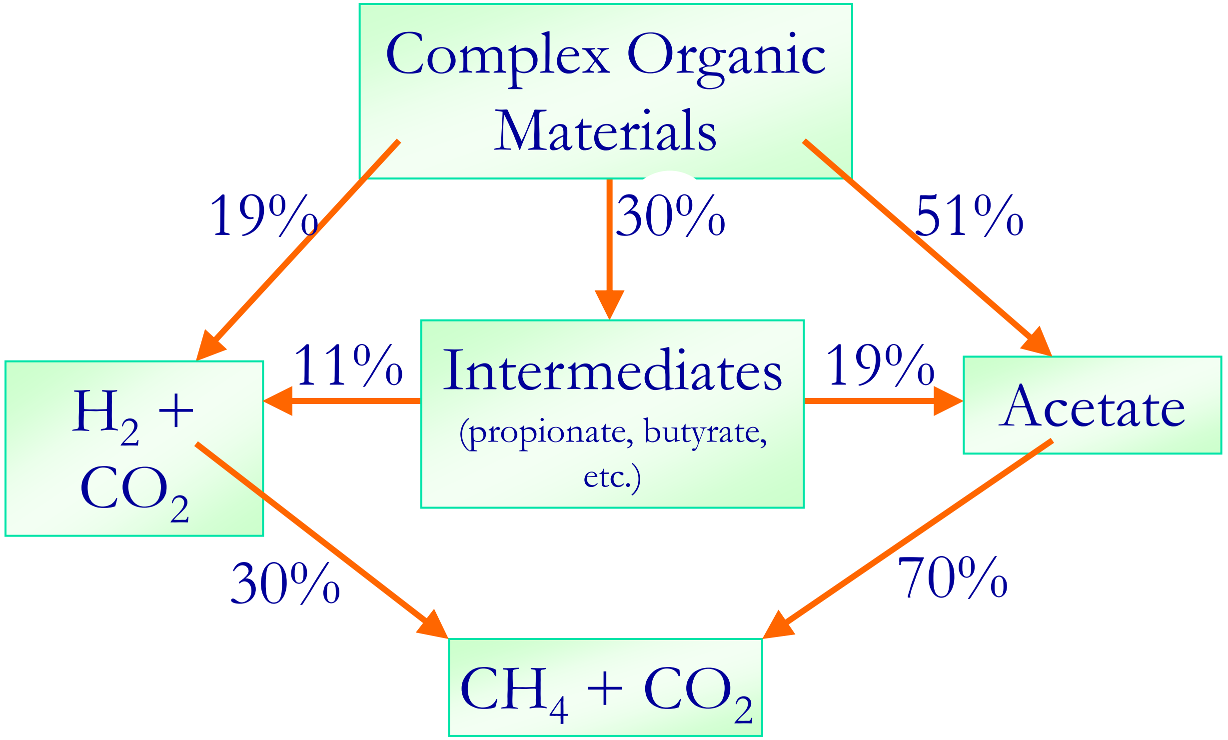
•* Thauer RK, Jungermann K, Decker K (1977) Bacteriol Rev 41:100

Anaerobic Digestion

Carbon flow in anaerobic environments with **active methanogens**



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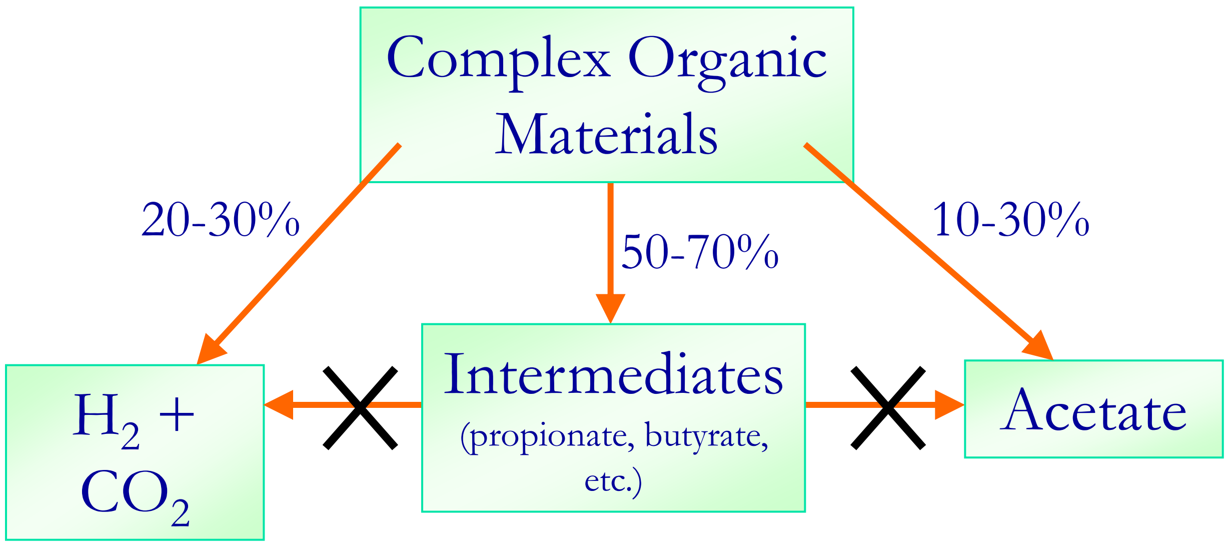
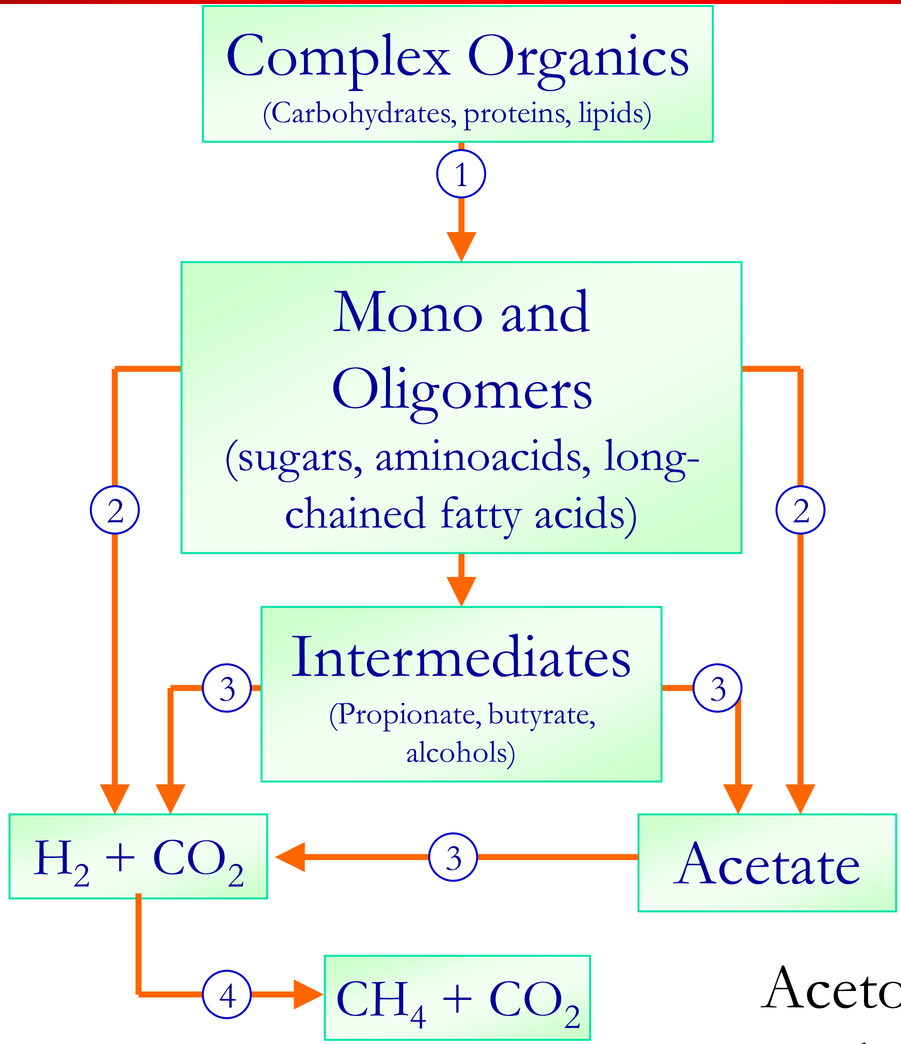
Anaerobic Digestion

Carbon flow in anaerobic environments <u>without</u> **active methanogens**



- **X**: Inhibited because of high H_2 partial pressure

Syntrophic Acetate Conversion



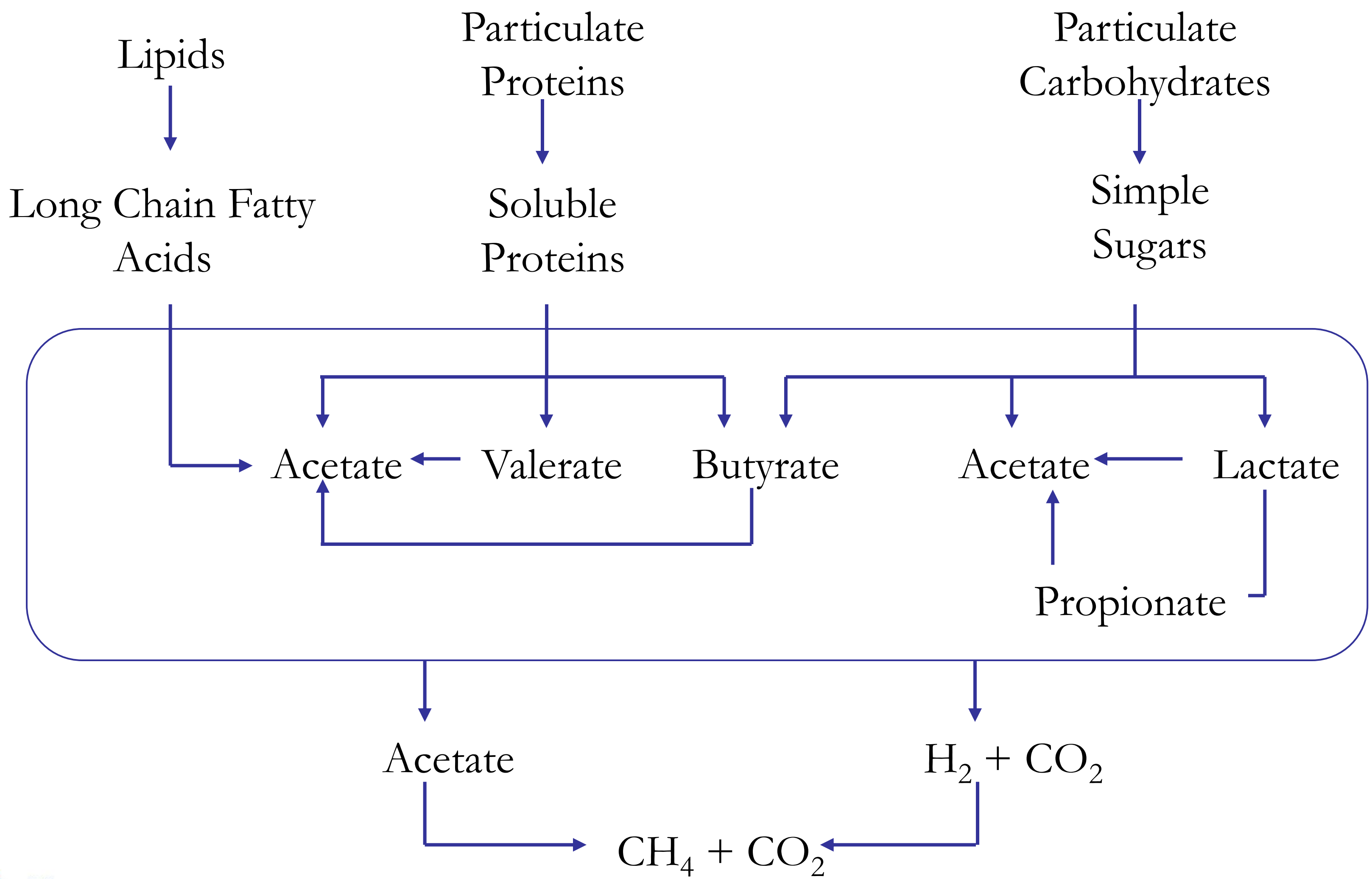
1. Hydrolysis
2. Fermentation
3. Acetogenesis
4. Methanogenesis

Acetoclastic methanogenesis is inhibited

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°C (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.

Biochemical Pathways



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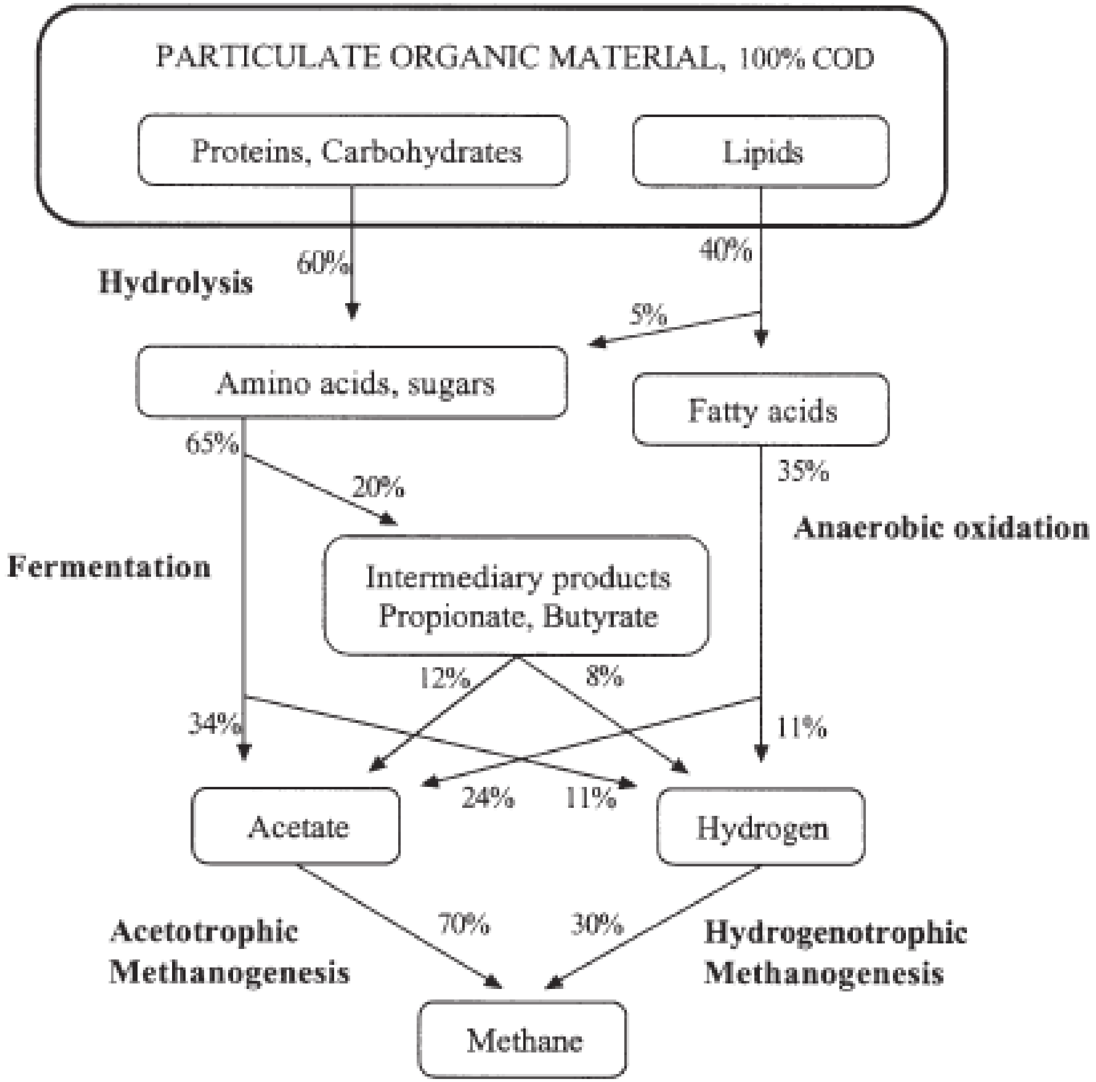
AD of Sewage Sludge



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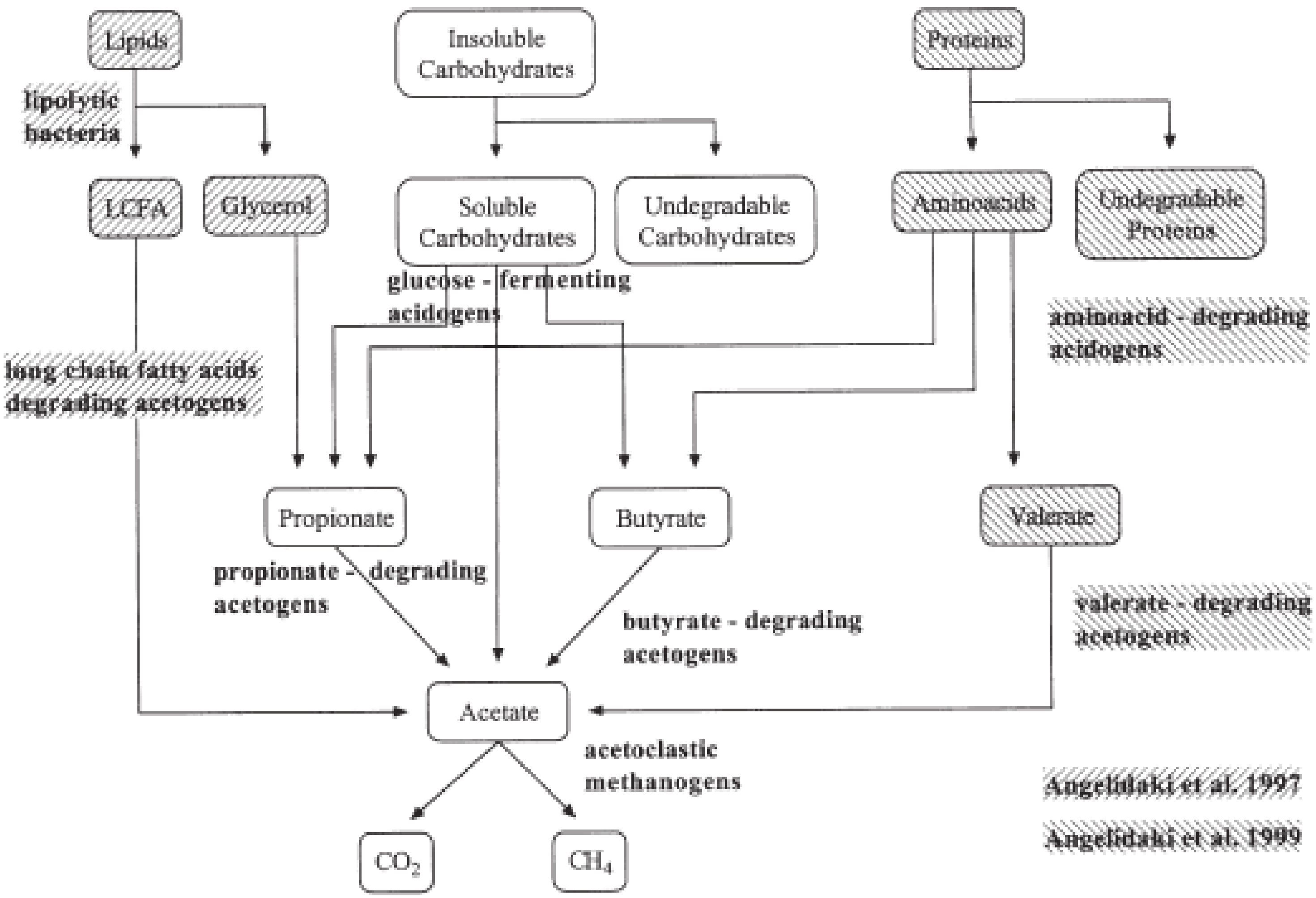
AD of Lipids & Proteins



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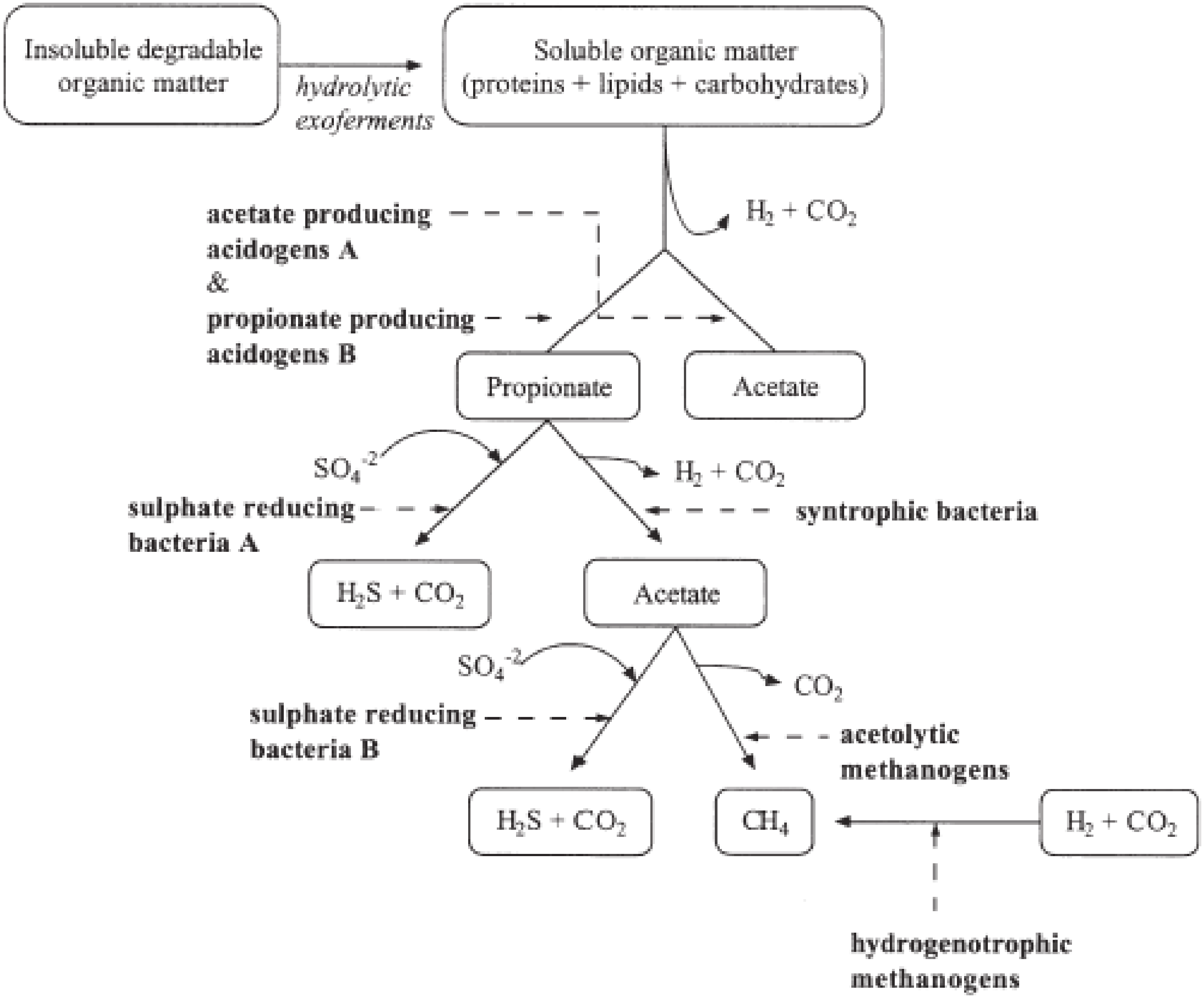
AD with Sulfate Reduction



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