Anaerobic Digestion: Microbiology and biochemistry

What is anaerobic digestion?

- Microbial degradation of organic material under anaerobic conditions
- Ubiquitous, naturally-occurring process
- Occurs in swamps, hydric soils, landfills, digestive tracks of ruminant animals and termites

Discovery of methane

- Alessandro Volta discovered methane in 1776 through studying swamp bottoms

- Volta experiment at Rutgers

How does AD work?

- Consortia of microorganisms work in a step-wise reaction that leads to production of methane
- Carbon in organic molecules is fully reduced to methane (CH_4).
- Functions through synergistic relationships between acid producing and acid consuming microorganisms.

Metabolic processes

- All metabolic processes requires electron donors (reducer) and acceptors (oxidizer)
- Aerobic organisms use oxygen as e^- acceptor
  - O_2 is reduced to CO_2
  - Generates greater ATP yield than anaerobic metabolism
- Anaerobic organisms must find alternatives e^- acceptors
  - NO^{3-}, Fe^{3+}, Mn^{3+}, SO_4^{3+}, CO_2

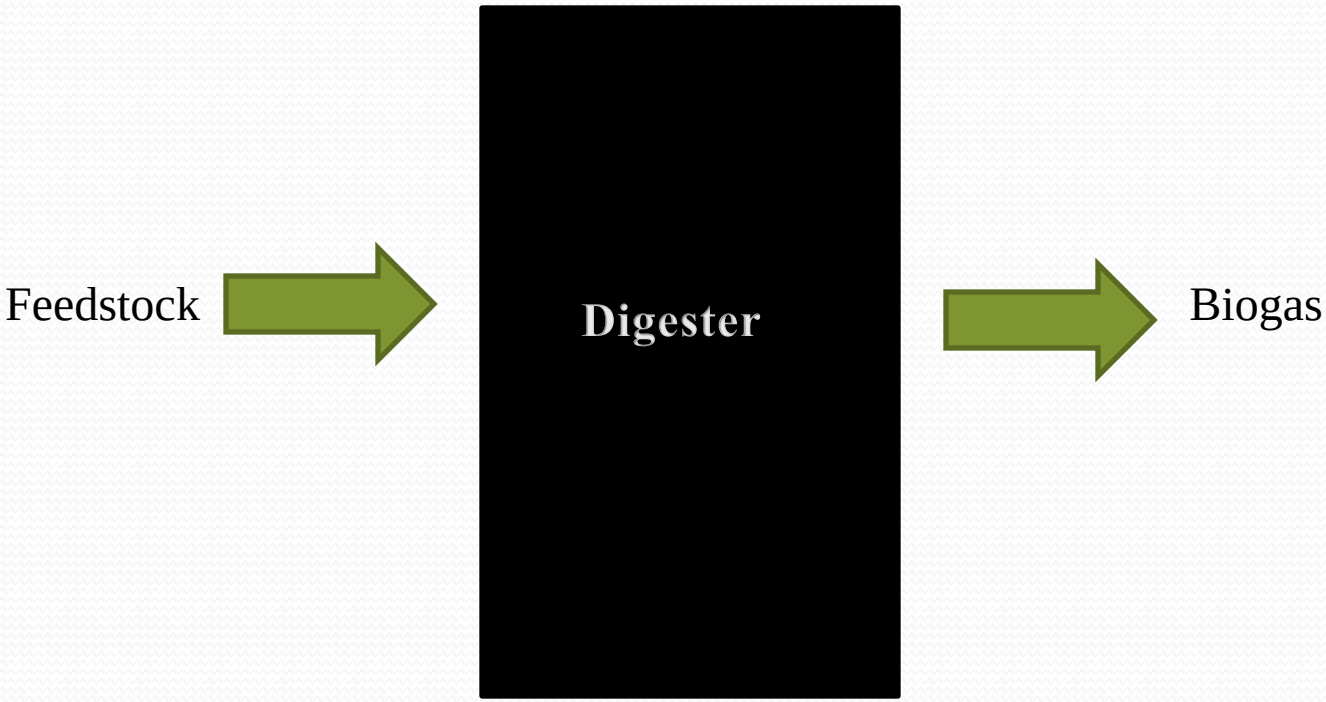
Anaerobic metabolic processes

- Anaerobic fermentation
  - Organic material is e^- donor
  - Internal cell products are e^- acceptors
  - Generates lower yield of ATP due to lack of electron transport chain
  - Can produce ethanol, acetone, organic acids, etc.
  - Performed by bacteria and fungi (i.e. yeast)

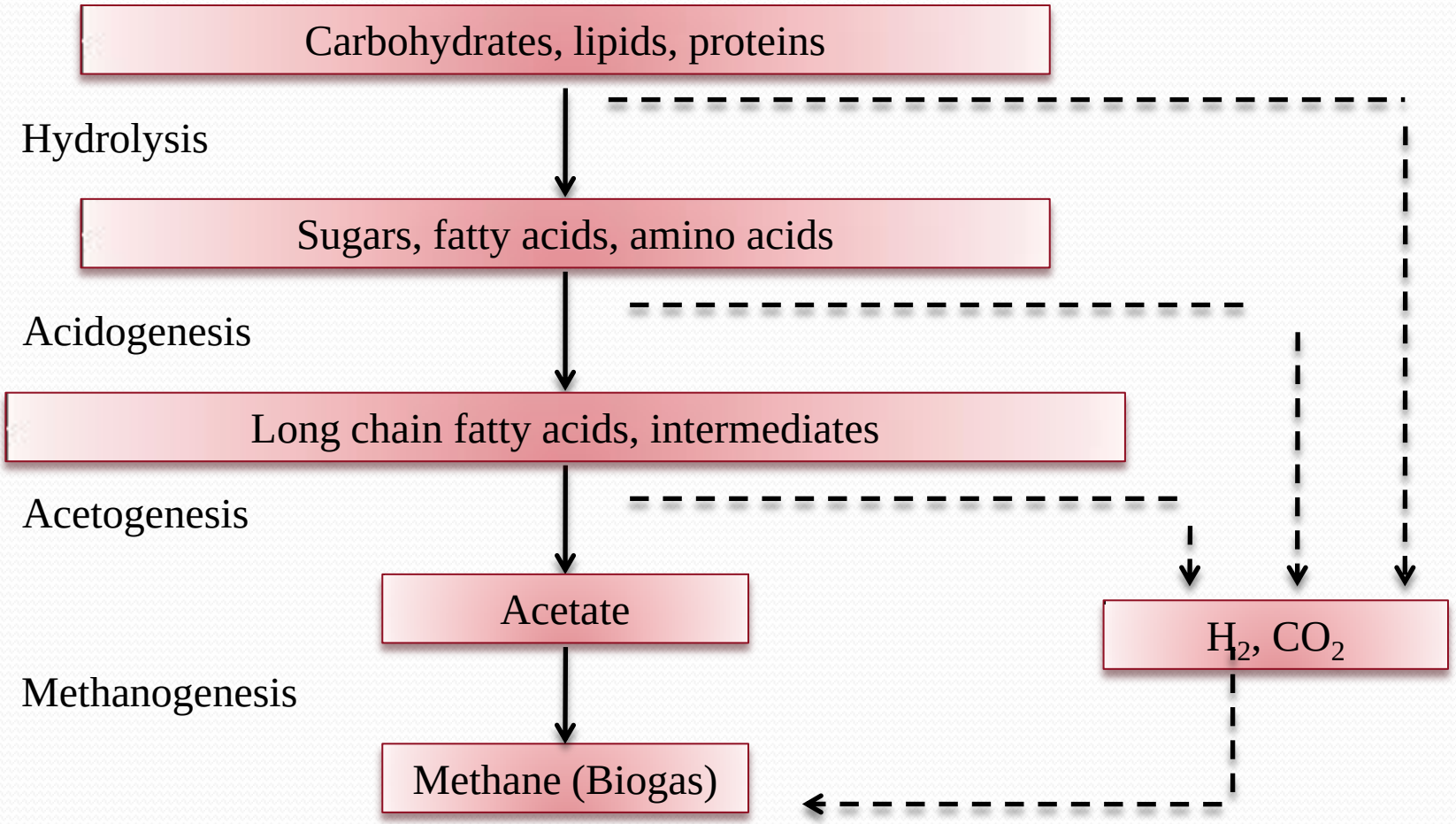
Anaerobic metabolic processes

- Anaerobic respiration
  - Uses organic compounds as e^- donor (at least in AD)
  - Requires external, alternative e^- acceptor (allows electron transport chain to function)
  - Generates reduced compounds (e.g. CH_4)
  - Predominantly performed by bacteria and *archaeabacteria*
- Both fermentation and respiration occur during AD

Digester is more than a black box...



…but an elegant microbial machine



Hydrolysis

- Large organic compounds are broken down into monomeric compounds
- Allows cell to assimilate materials
- Performed by many organisms
  - Bacteria, fungi, protists

Pseudomonas sp.

Hartmanella sp.

Carbohydrates, lipids, proteins

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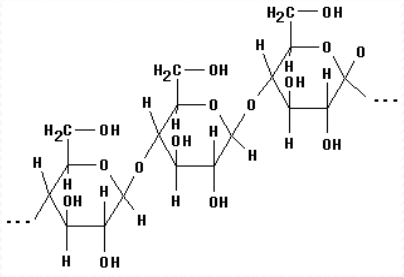
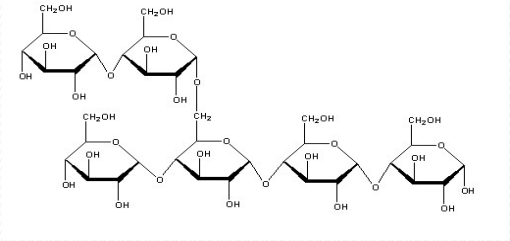
Sugars, fatty acids, amino acids

Hydrolysis

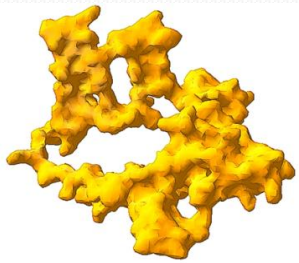
- Accomplished through extracellular enzymes



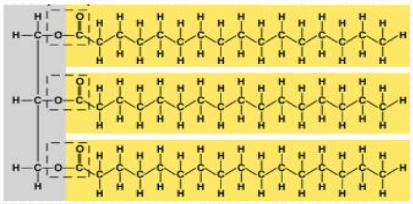
Cellulose



Starch



Casein



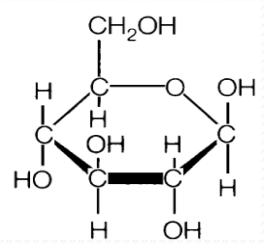
Triglyceride

Cellulases

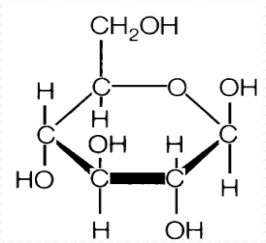
Amylases

Proteases

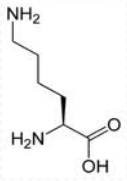
Lipases



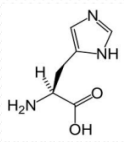
Glucose



Glucose

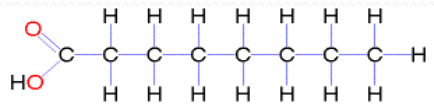


Lysine



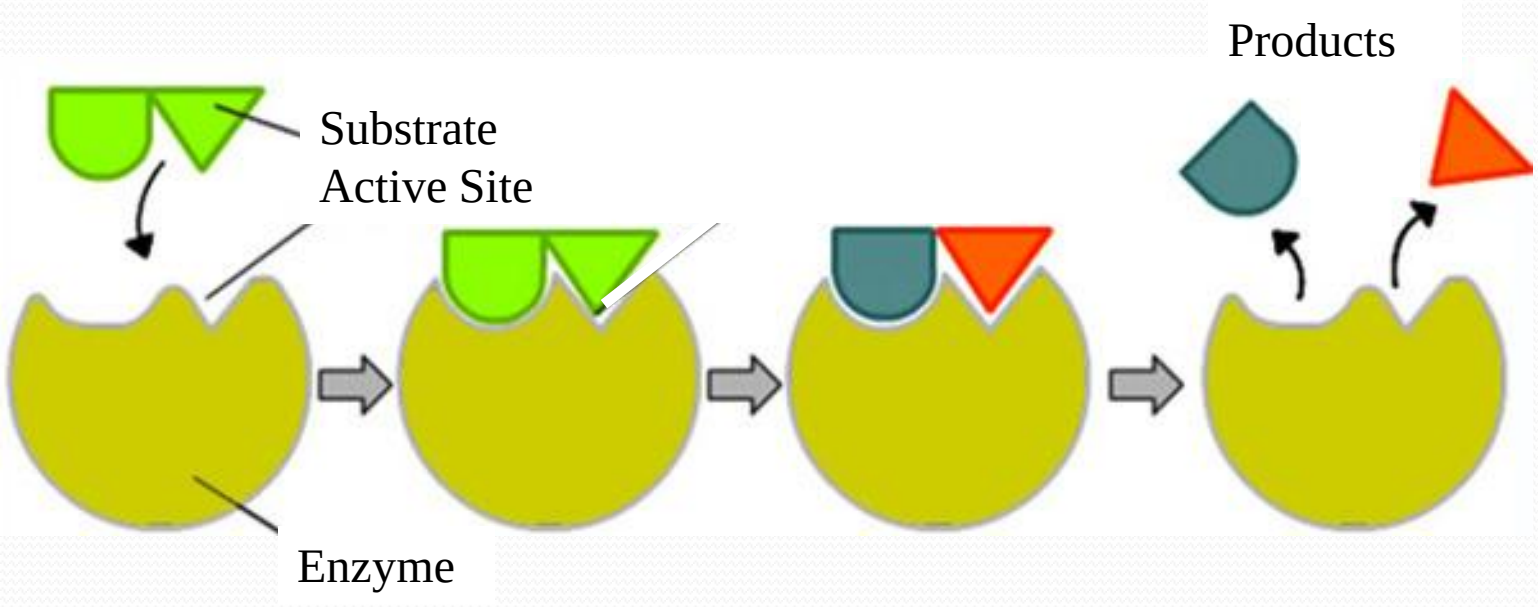
Histidine

Amino Acids



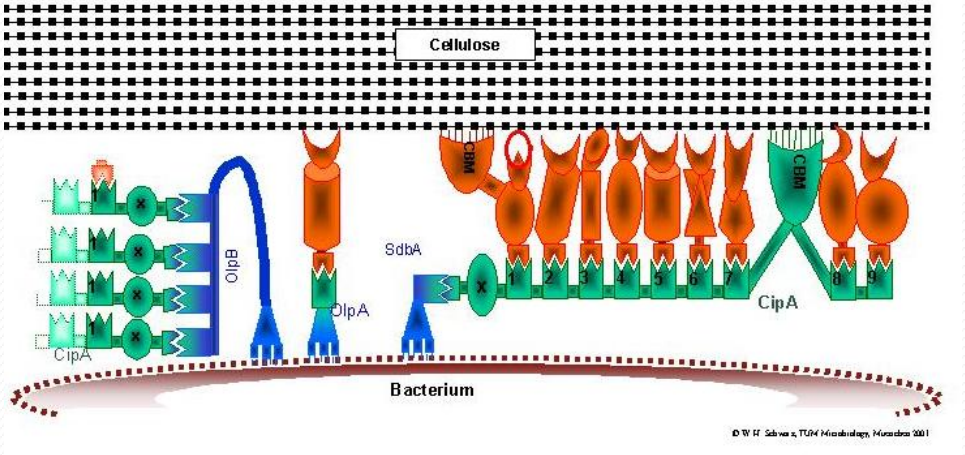
Fatty Acid

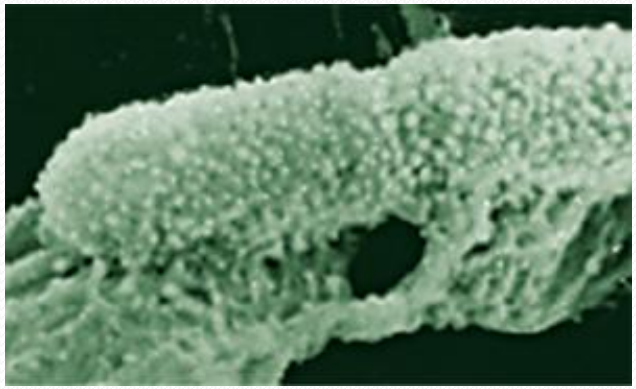
Hydrolytic enzyme action



Hydrolysis

- Some bacteria produces cellular scaffolding to attach cell to substrate
- Example: Cellulosome – contains cellulases to cleave bonds between glucoses in the cellulose molecule

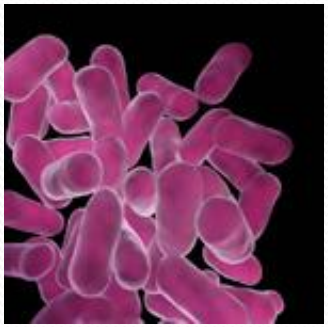




Acetovibrio cellulyticus

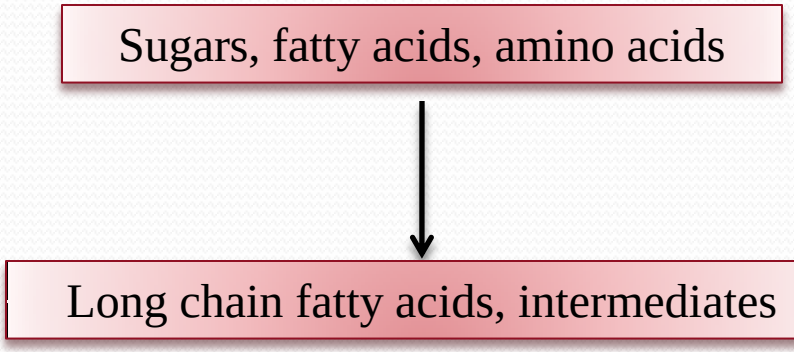
Acidogenesis

- Monomeric products hydrolysis are fermented into fatty acid intermediaries
- Performed by acidogens
- Generally fastest step in process
- Unbalanced acidogenesis can cause acidification



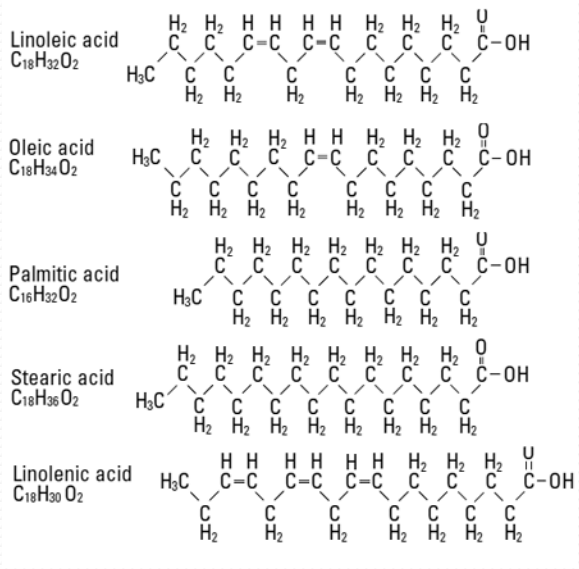
Lactobacillus sp.

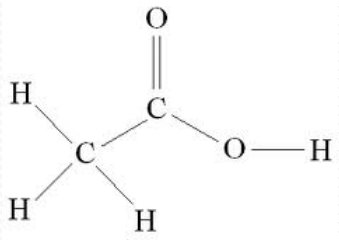
Propionibacterium sp.



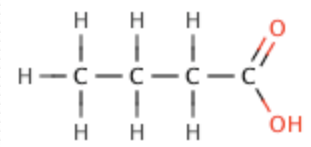
Acidogenesis

- Many different fatty acids produced
  - Long-chain fatty acids (LCFAs) (generally produced from lipids)
  - Volatile fatty acids(VFAs) (butyric, proprionic, acetic acid)

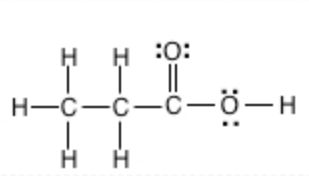




Acetic Acid



Butyric acid

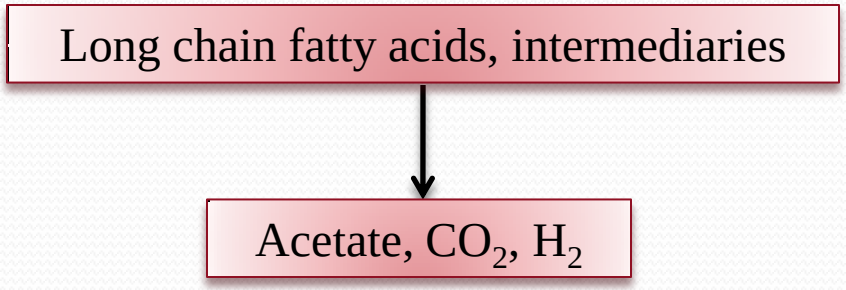


Proprionic acid

Acetogenesis

- Acetate production is critical to AD
- Immediate precursor for majority of methane production
- Some acetate is produced through direct fermentation (i.e. mixed-acid fermentation)
- Most is through secondary fermentation
  - Converts intermediaries (proprionate, butyrate, etc.) to acetate

Acetobacter sp.

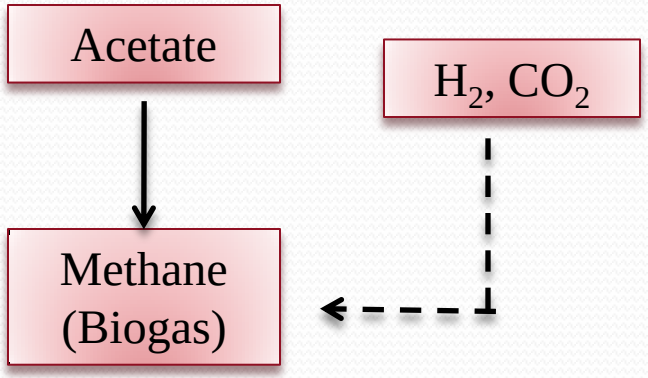


Acetogenesis

- Two groups of acetogens
  - Obligate hydrogen-producing acetogens (OHPA) – more dominant, produce acetate, CO_2 and H_2 from fatty acids
  - Homoacetogens – less dominant, converts CO_2 and H_2 to acetate through anaerobic respiration

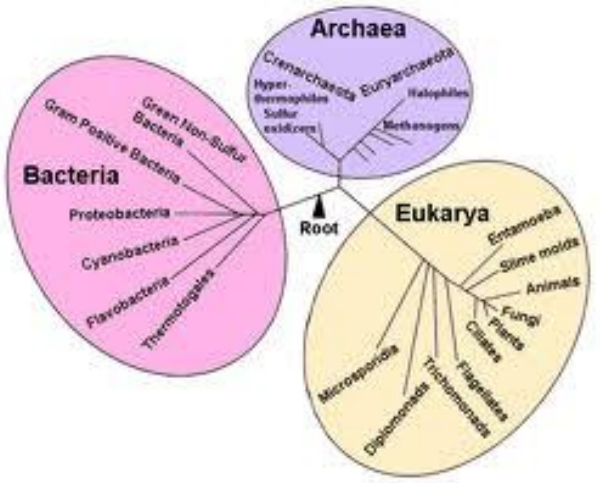
Methanogenesis

- Final metabolic phase in AD
- Methanogens produce methane gas from acetate or H_2 and CO_2
- 2/3 of methane produced is derived from acetate
- Often the rate limiting step in anaerobic digestion of acids



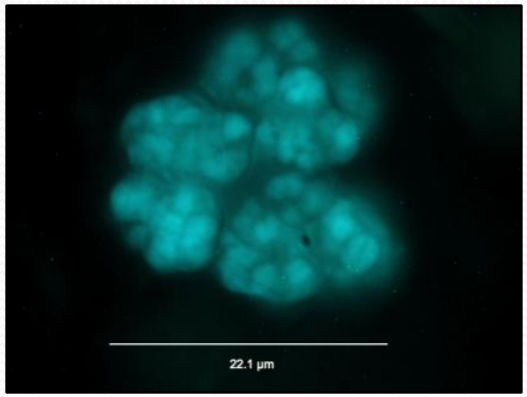
Methanogens

- All are in Archae domain
- Ancient organisms, contain many unique co-enzymes (e.g. f420)
- Obligate anaerobes, but can form in biofilms and granules for aerobic protection
- Optimum performance at neutral pH
- Two main groups: acetoclastic and hydrogenotrophic

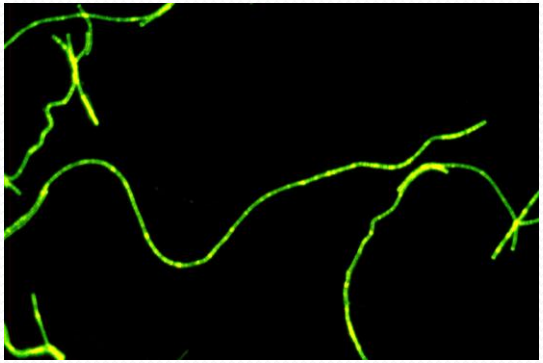


Methanogens

- Two known genera convert acetate to methane (Acetoclastic)
  - Methanosarcina – Favors high concentration
  - Methanosaeta – Favors low concentration
- Also produces CO2 as metabolic by-product



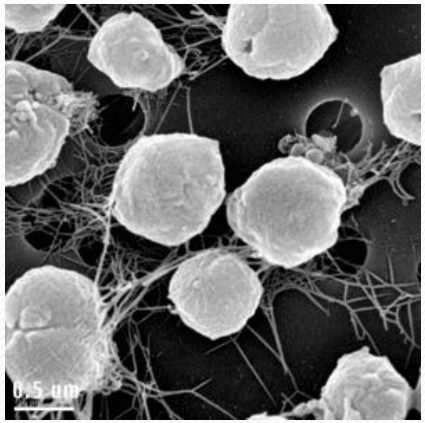
Methanosarcina sp.



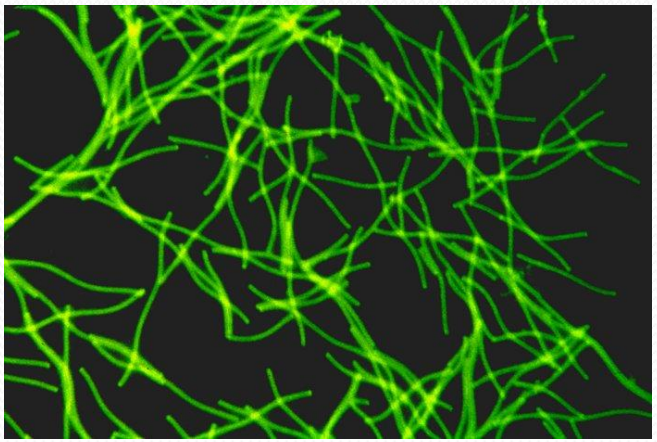
Methanosaeta sp.

Methanogenesis

- Hydrogenotrophic methanogens
  - Produces methane from H_2 and CO_2
  - Less dominant in AD
  - Mediates syntropy with acetogens



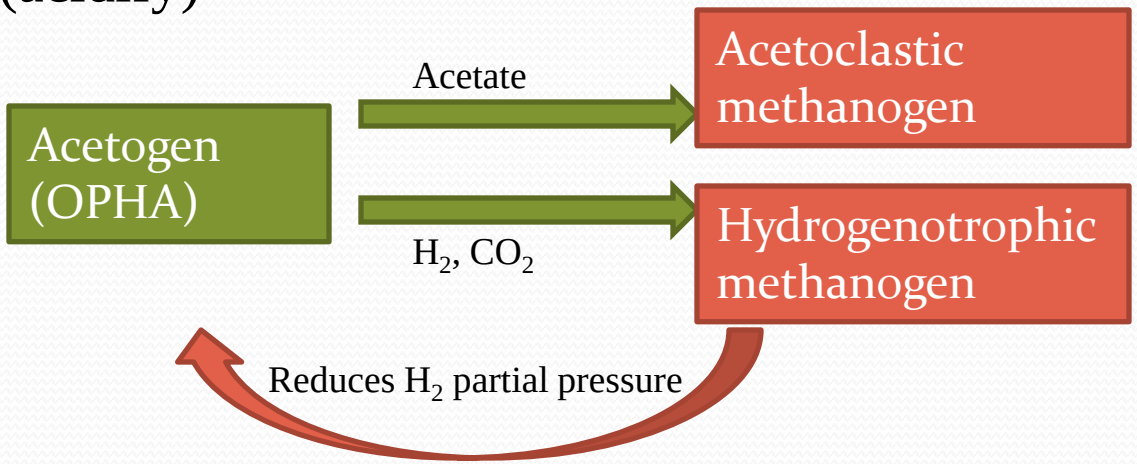
Methanococcus sp.



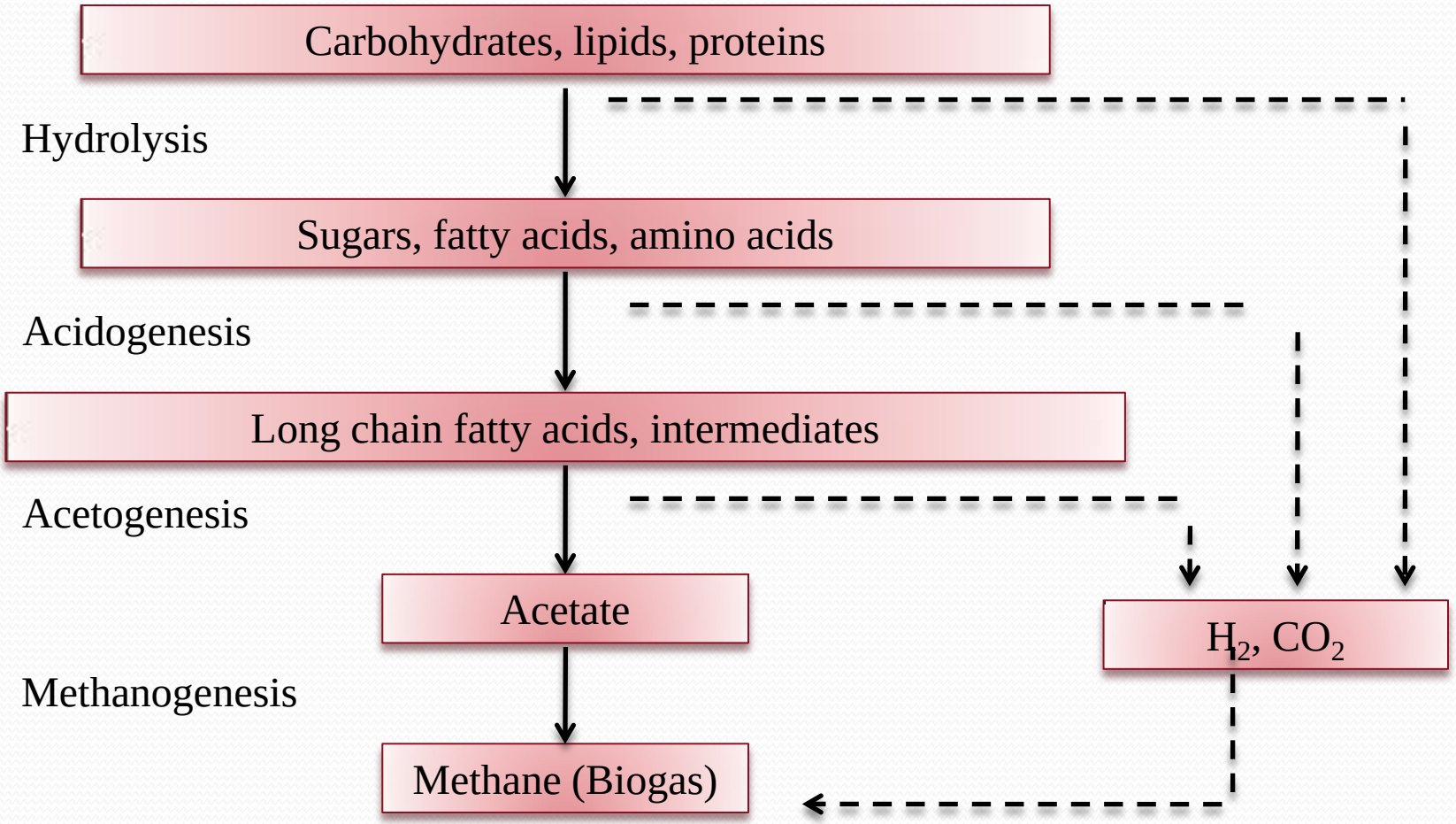
Methanospirillum sp.

Hydrogen syntropy in anaerobic digestion

- Acetate production from OPHA inhibited at high partial pressure of H_2
- Prevents conversion of acid intermediaries to acetate and further consumption through methanogenesis
- Hydrogenotrophic methanogens consume H_2, which reduces H_2 partial pressure
- Methanogens and OPHA live in close mutualistic relationship
- Without syntropy, intermediates would accumulate and system would crash (acidify)



Questions?


Carbohydrates, lipids, proteins
Hydrolysis
Sugars, fatty acids, amino acids
Acidogenesis
Long chain fatty acids, intermediates
Acetogenesis
Acetate
H_2, CO_2
Methanogenesis
Methane (Biogas)