

# Anaerobic Digestion

Microbiological perspective

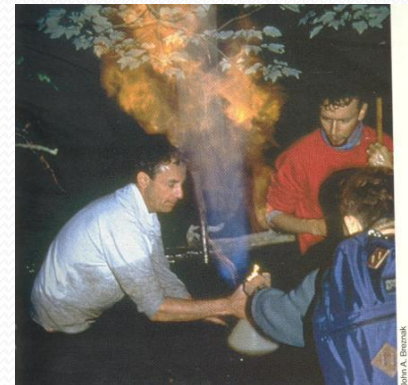
# What is anaerobic digestion?

- Microbial degradation of organic waste under anaerobic conditions
- Ubiquitous, naturally-occurring process
- Found in swamps, hydric soils, landfills, rumens



# Discovery of methane

- Alessandro Volta discovered methane in 1776 through studying swamp bottoms
- This video demonstrates how the power of methane was first discovered.
  - [Volta experiment at Rutgers](#)



# How does AD work?

- Consortia of bacteria and microbes work in a serial reaction that leads to production of methane
- Products of one phase are feedstocks for the next
- Overall process only as fast as its slowest step

# Anaerobic metabolic processes

- All metabolism requires electron donor (reducer) and acceptors (oxidizer)
- Aerobic organisms use oxygen as  $e^-$  acceptor (high ATP yield)
- Anaerobic organisms must find alternatives  $e^-$  acceptors
  - $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{3+}$ ,  $\text{SO}_4^{3+}$ ,  $\text{CO}_2$

# Anaerobic metabolic processes

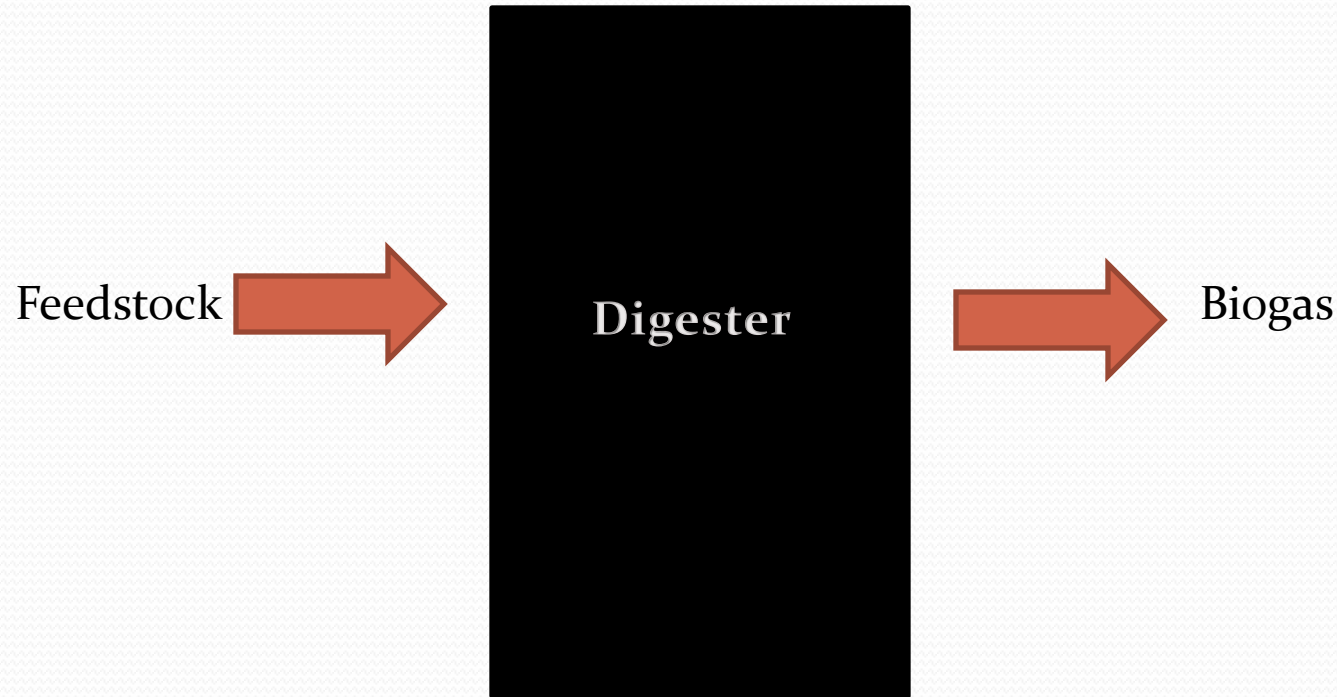
- Anaerobic fermentation
  - Organic material is  $e^-$  donor
  - Internal cell products are  $e^-$  acceptors
  - Generates lower yield of ATP
  - Can produce ethanol, butanol, acetone, acetic acid, etc.
  - Performed by bacteria and fungi (i.e. yeast)

# 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 *archaea*
- Both fermentation and respiration occur during AD

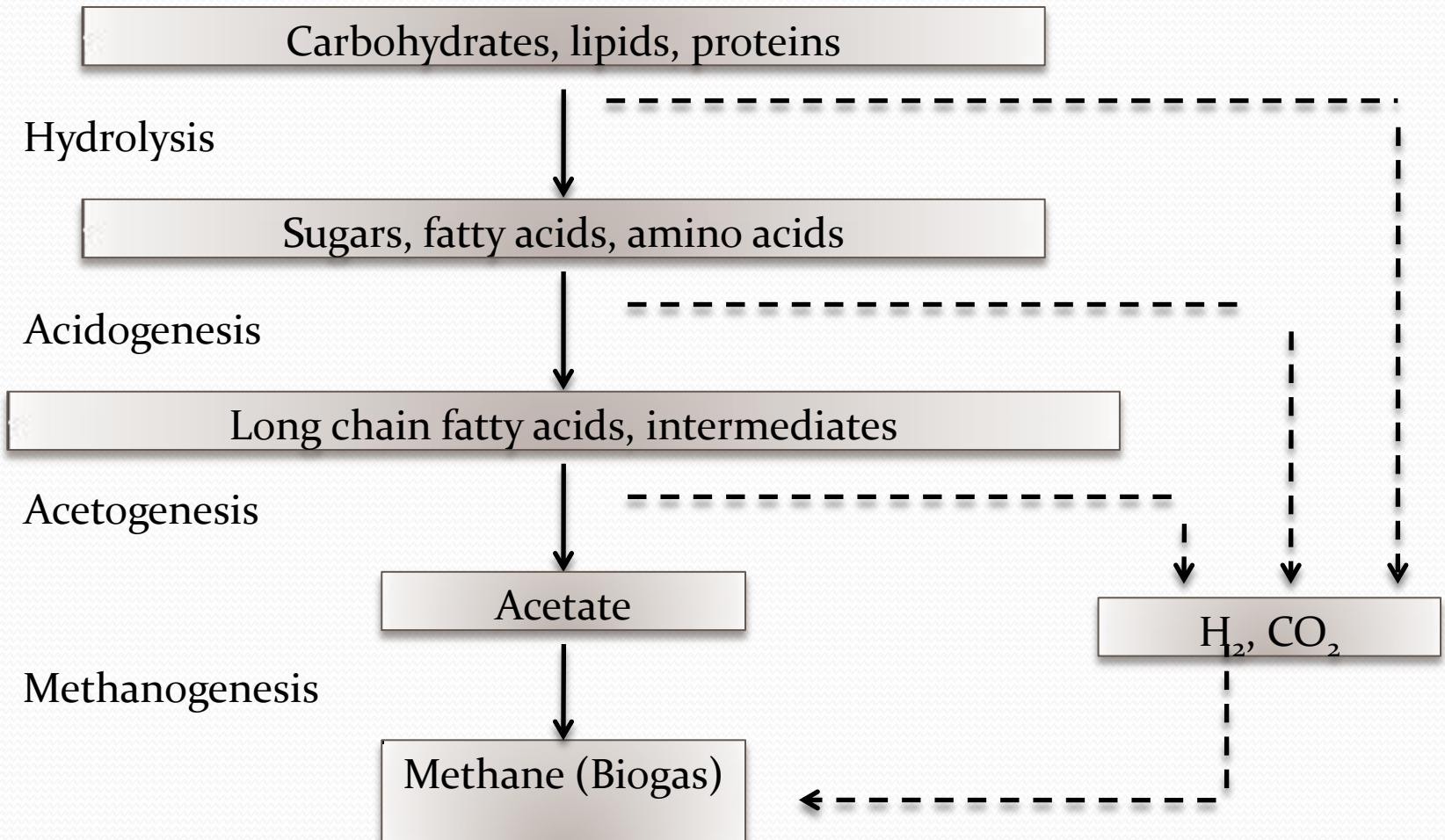
# AD is much more elaborate than people think

Not just a black box...



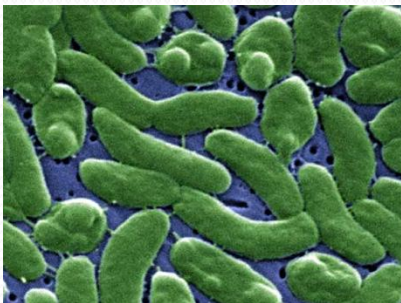


# ...but an elegant microbial machine



# Hydrolysis

- Large organic compounds are broken down into simpler compounds
- Allows cell to take-in materials
- Performed by many organisms
  - Bacteria (including acidogens), fungi, protists



Vibrio



Hartmanella

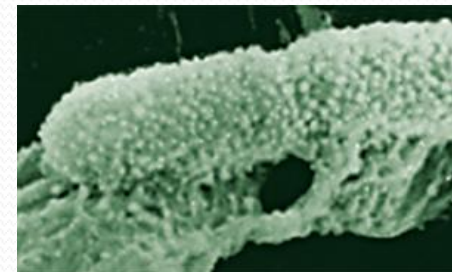
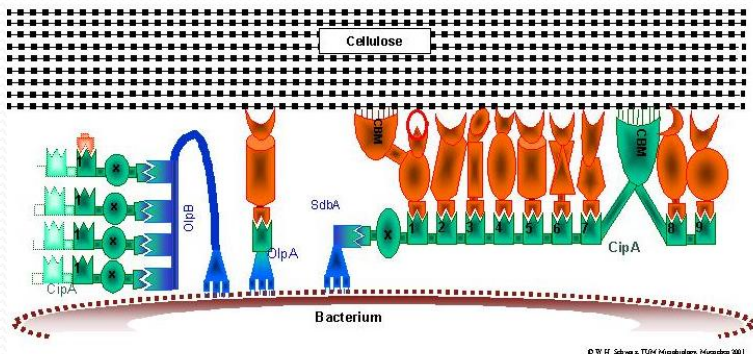
Carbohydrates, lipids, proteins



Sugars, fatty acids, amino acids

# Hydrolysis

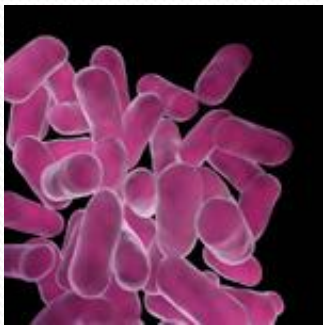
- Processes uses extracellular enzymes (cellulases, amylases, lipases, proteases)
- Important for both AD and cellulosic ethanol
- Particular interest on cellulosomes
  - Extracellular structures bearing cellulases
  - Allows cell to attach and break cellulose structure



*Acetovibrio cellulyticus*

# Acidogenesis

- Products of hydrolysis are fermented into fatty acid intermediaries
- Performed by acidogens
- Generally fastest step in process
- Unbalanced acidogenesis can cause acidification
- Trace oxygen consumed by facultative bacteria



Lactobacillus



Propionibacterium

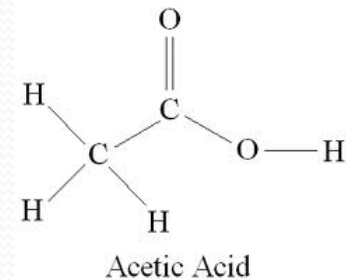
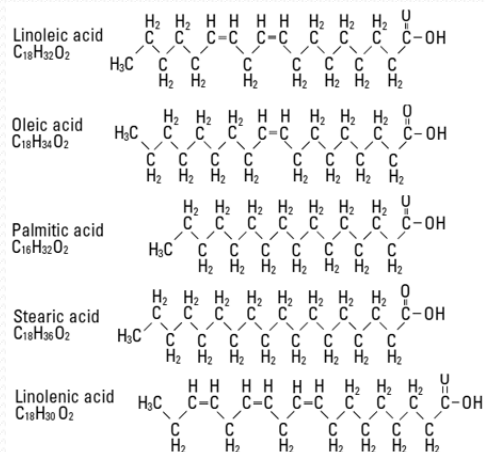
Sugars, fatty acids, amino acids



Long chain fatty acids, intermediates

# Acidogenesis

- Many different fatty acids produced
  - Long-chain fatty acids (LCFAs) (generally produced from lipids)
  - Volatile fatty acids (VFAs) (butyric, propionic, acetic acid), very important in AD



# Acetogenesis

- Acetate is very important in AD
- Immediate precursor for majority of methane production
- Some acetate is produced through direct fermentation (mixed acid fermentation)
- Most is through secondary fermentation
  - Converts intermediaries (propionate, butyrate) to acetate



*Syntrophomonas*

Long chain fatty acids, intermediaries



Acetate, CO<sub>2</sub>, H<sub>2</sub>

# Acetogenesis

- Two groups of acetogens
  - Obligate hydrogen-producing acetogens (OHPA) – more dominant
  - Homoacetogens – less dominant, converts  $\text{CO}_2$  and  $\text{H}_2$  to acetate through respiration

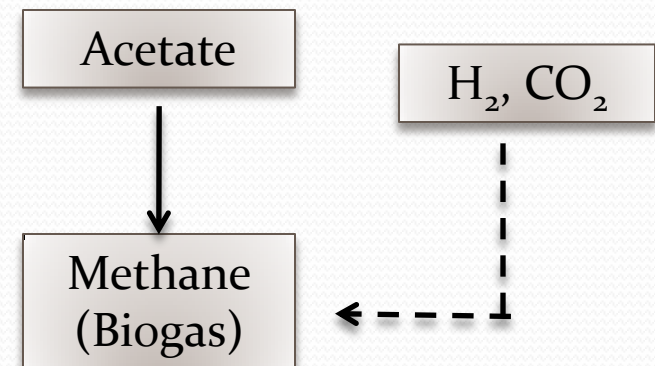
# Acetogenesis (Hydrogen syntrophy)

- OHPA produce acetate,  $\text{CO}_2$  and  $\text{H}_2$  from fatty acids
- Acetate production inhibited at high partial pressure of  $\text{H}_2$
- Forms mutualistic relationship with hydrogen-consuming methanogens (syntrophy)
  - Methanogens consume  $\text{H}_2$ , reducing  $\text{H}_2$  partial pressure to for acetogens
- Homoacetogens can also assist in  $\text{H}_2$  reduction



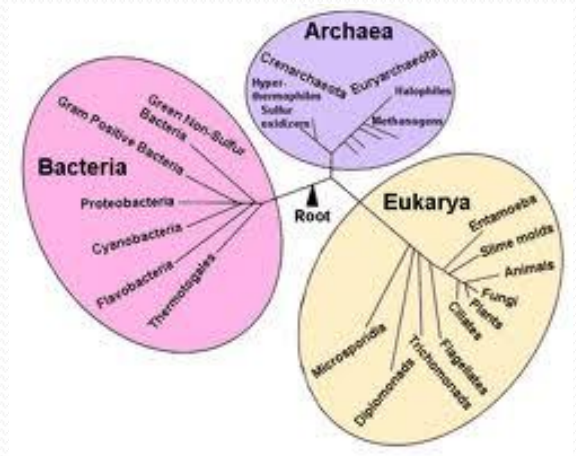
# Methanogenesis

- 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



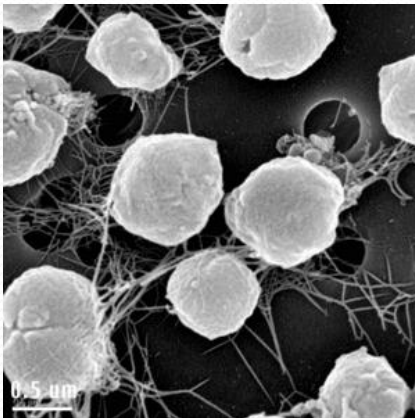
# Methanogens

- All are in Archaeae domain
- Obligate anaerobes, but can form in biofilms and granules for aerobic protection
- Optimum performance at neutral pH (7)
- Two main groups: hydrogenotrophic and acetoclastic

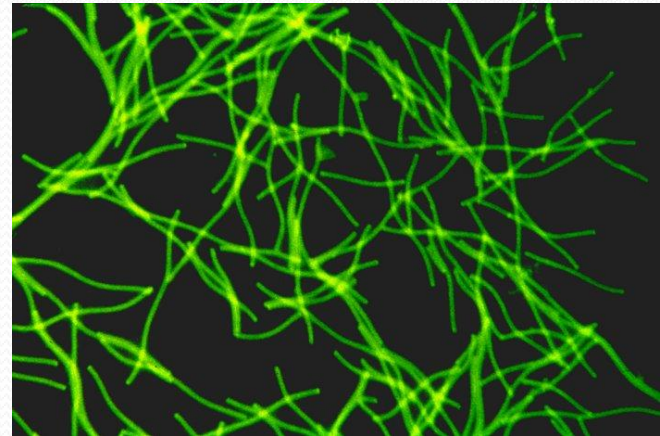


# Methanogenesis

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



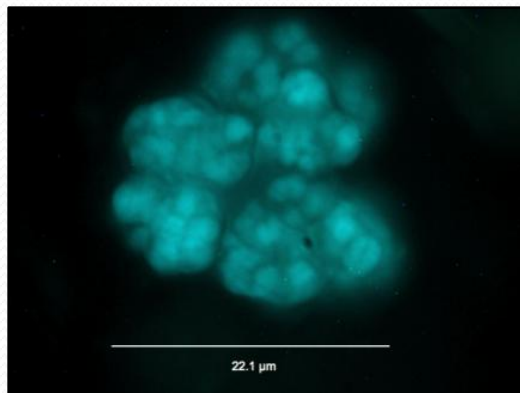
*Methanococcus*



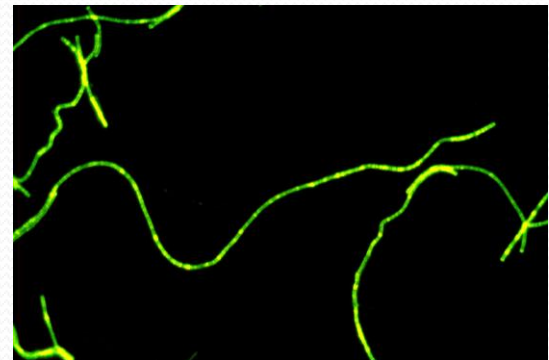
*Methanospirillum*

# Methanogens

- Two known genera convert acetate to methane (Acetoclastic)
  - Methanosarcina – Favors high concentration
  - Methanosaeta – Favors low concentration
- Also produces CO<sub>2</sub> as metabolic by-product



*Methanosarcina* sp.



*Methanosaeta* sp.

# Questions?

