Università
Ca'Foscari
Venezia

Dipartimento
di Scienze Ambientali
Informatica e Statistica

ANAEROBIC DIGESTION FUNDAMENTALS I

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LECTURE 1

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UNIVERSITY OF JYVÄSKYLÄ

Summer School on Biogas Technology for sustainable Second Generation Biofuel Production, 15-19 August 2011

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….200 years ago…..

In November 1776, Alessandro Volta performed his classic experiment disturbing the sediment of a shallow lake, collecting the gas and demonstrating that this gas was flammable.

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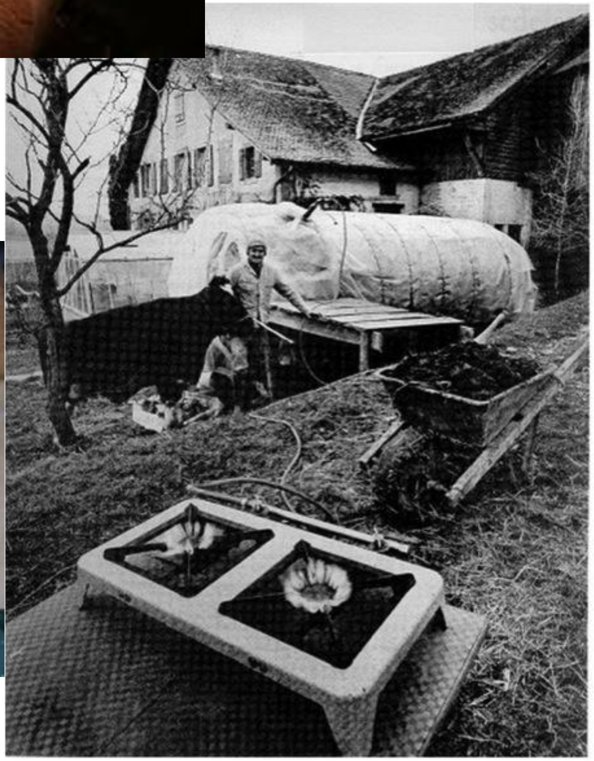
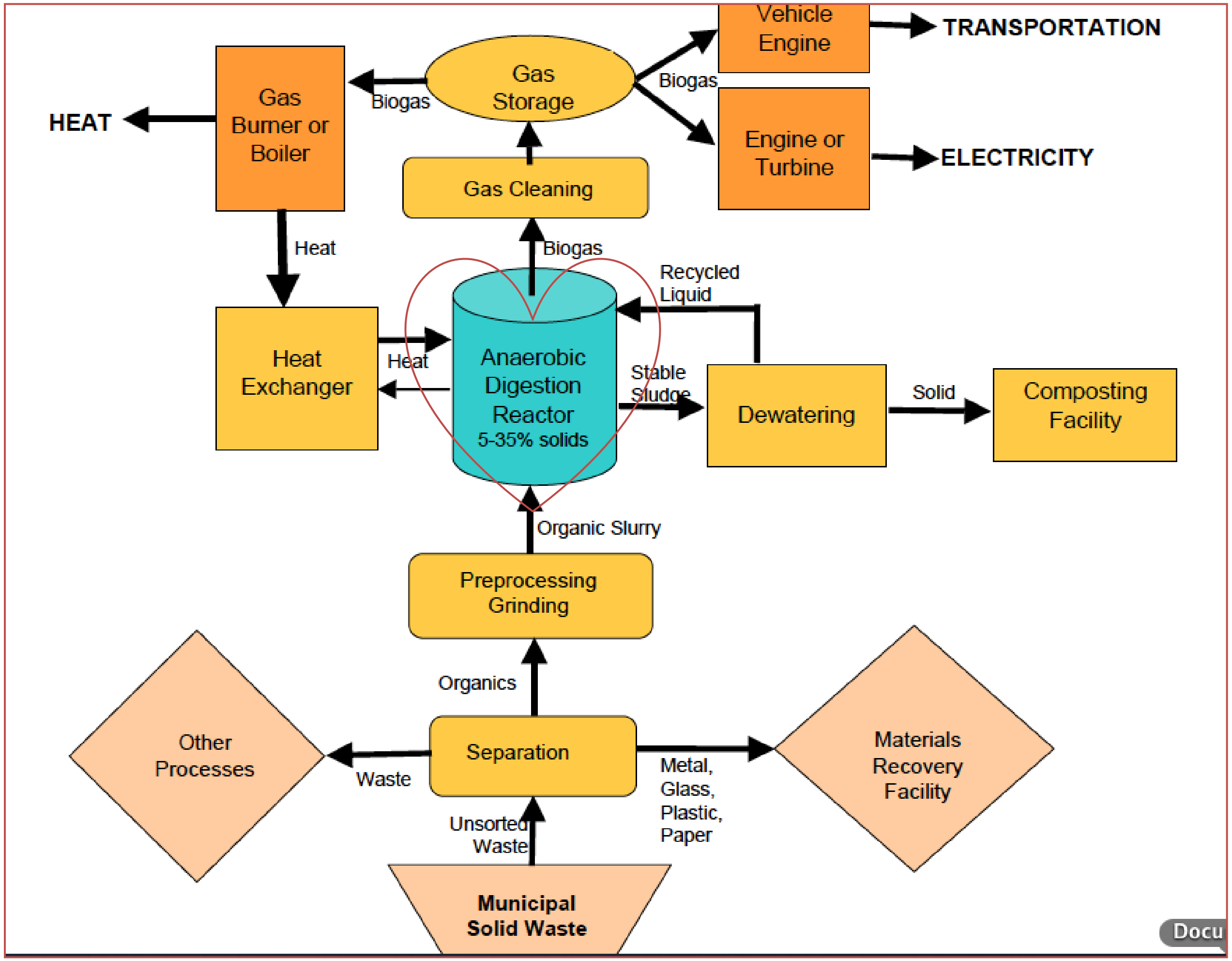
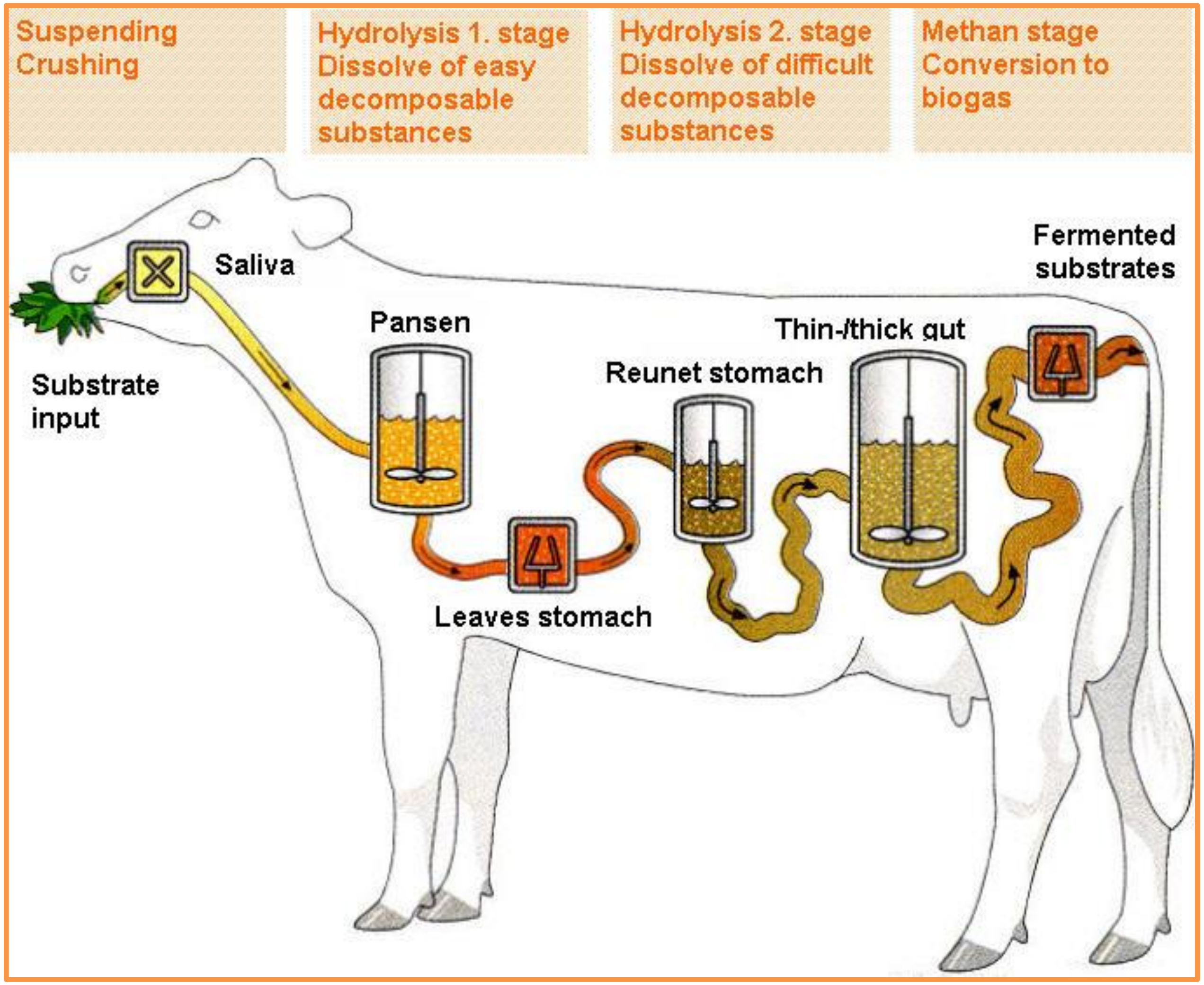
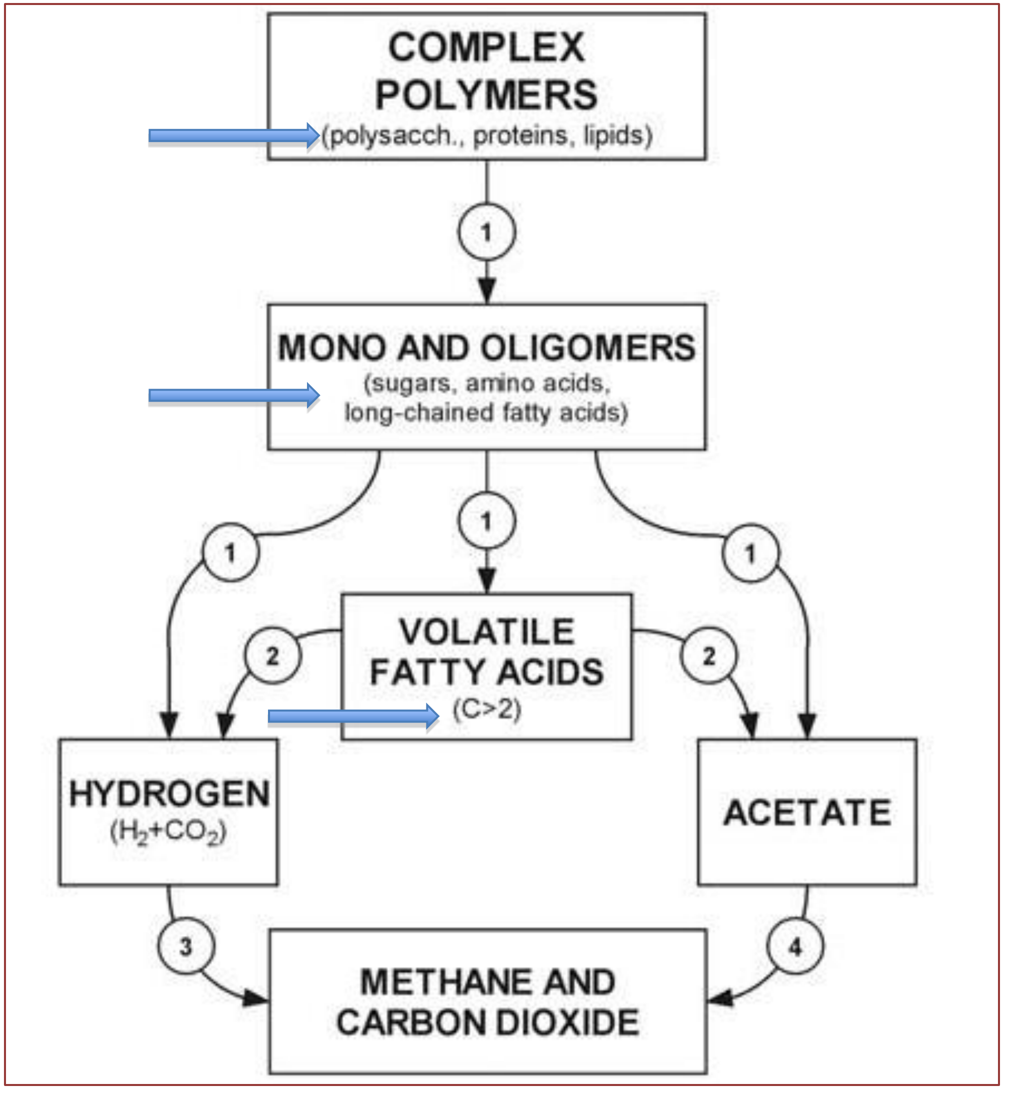
- First plant built in 1859 at leper colony in India
- Used in 1895 to power streetlights in Britain
- First used for Municipal Solid Waste in US in 1939
- Thousands of 'backyard' digesters throughout China, India other Asian countries
- Most sewage treatment works in Europe stabilize their sludge using AD
- Increasingly being used in Europe to manage municipal waste and to create heat and power




Vehicle Engine
TRANSPORTATION
Gas Storage
Biogas
Gas Burner or Boiler
HEAT
Biogas
Engine or Turbine
ELECTRICITY
Gas Cleaning
Heat
Biogas
Recycled Liquid
Heat Exchanger
Heat
Anaerobic Digestion Reactor
5-35% solids
Stable Sludge
Dewatering
Solid
Composting Facility
Organic Slurry
Preprocessing Grinding
Organics
Other Processes
Waste
Separation
Metal, Glass, Plastic, Paper
Materials Recovery Facility
Unsorted Waste
Municipal Solid Waste
Docu


Suspending
Crushing
Hydrolysis 1. stage
Dissolve of easy
decomposable
substances
Hydrolysis 2. stage
Dissolve of difficult
decomposable
substances
Methan stage
Conversion to
biogas
Saliva
Fermented
substances
Pansen
Thin-/thick gut
Reunet stomach
Substrate
input
Leaves stomach

Anaerobic Digestion consists of a series of bacterial events that convert organic compounds to methane, carbon dioxide, and new bacterial cells. These events are commonly considered as a three-stage process.



(Ahring, 2003)

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Different consortia of microorganisms with different function in the anaerobic digestion process are needed.

Three major groups of microorganisms have been identified with different functions in the overall degradation process:

- The hydrolyzing and fermenting microorganisms
- The obligate hydrogen-producing acetogenic bacteria
- Two groups of methanogenic Archaea

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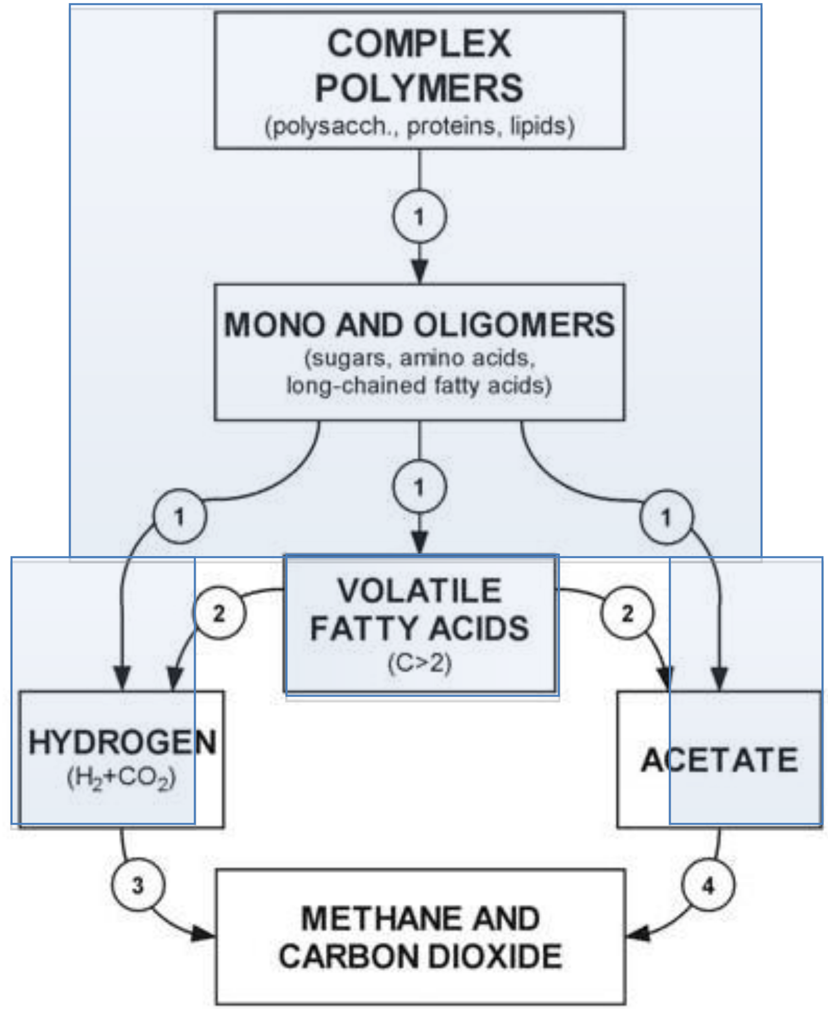
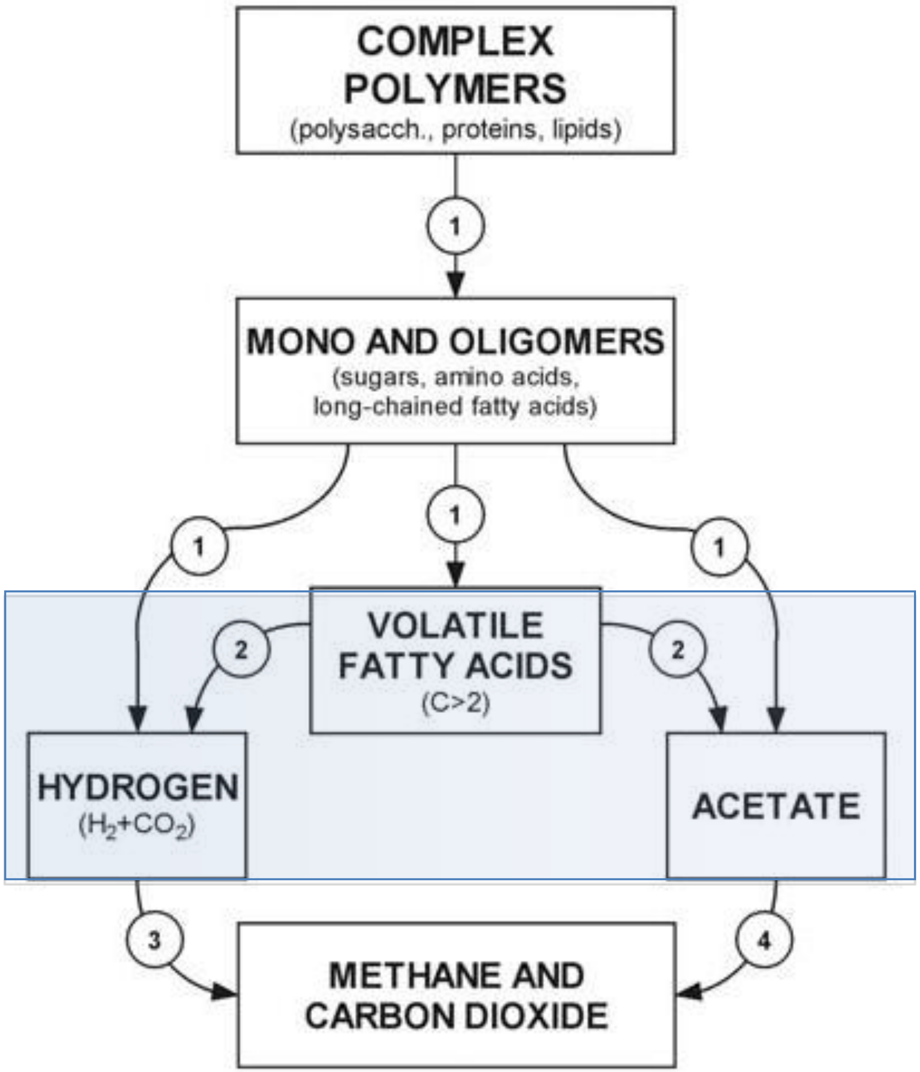
The hydrolyzing and fermenting microorganisms

Are responsible for the initial attack on polymers and monomers found in the waste material and produce mainly acetate and hydrogen, but also varying amounts of volatile fatty acids (VFA) such as propionate and butyrate as well as some alcohols.



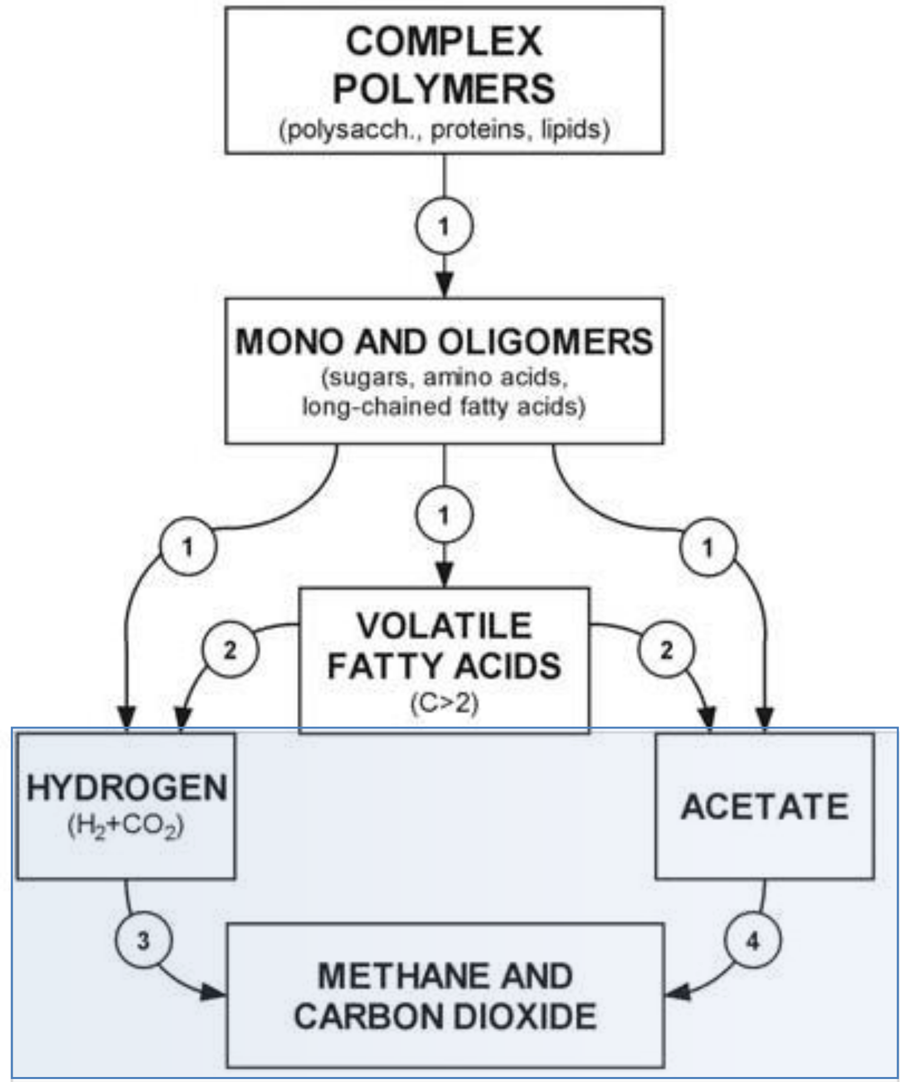
The obligate hydrogen-producing acetogenic bacteria

convert propionate
and butyrate into acetate and hydrogen.



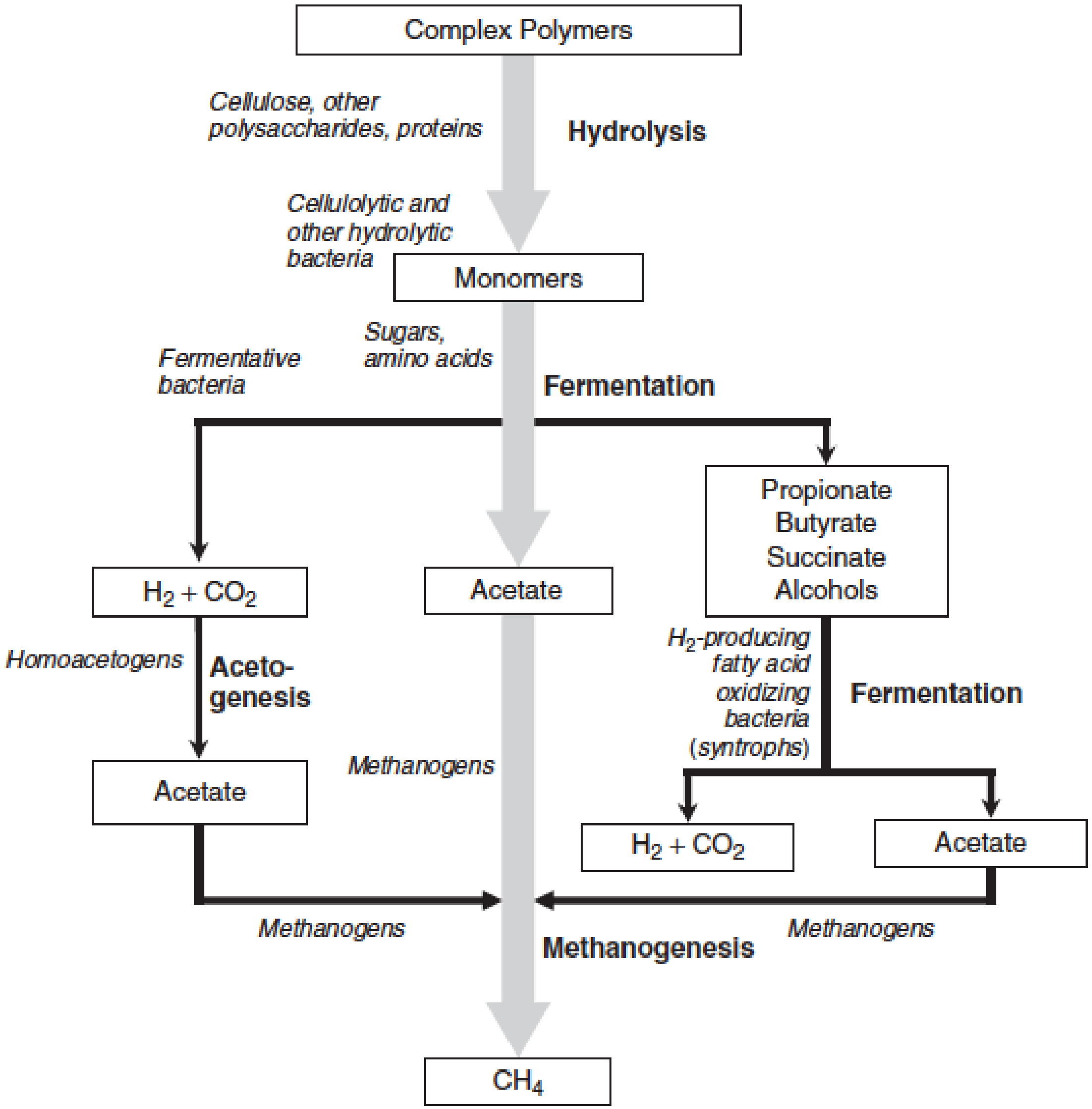
Two groups of methanogenic Archaea

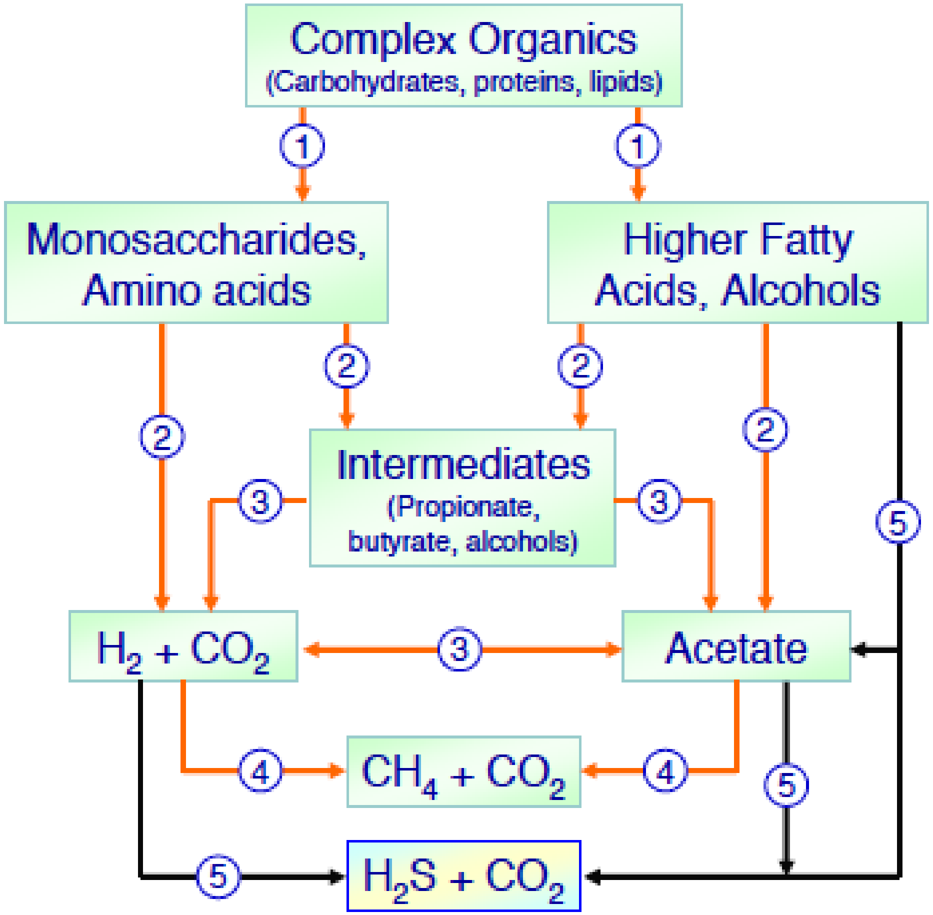
produce methane from acetate or hydrogen, respectively.



AD is a 'series' process, disruption of one part of the process disrupts the whole process.

Process rate proceeds at the rate of the slowest step.





1 Hydrolytic bacterIa

2 Fermentative bacteria

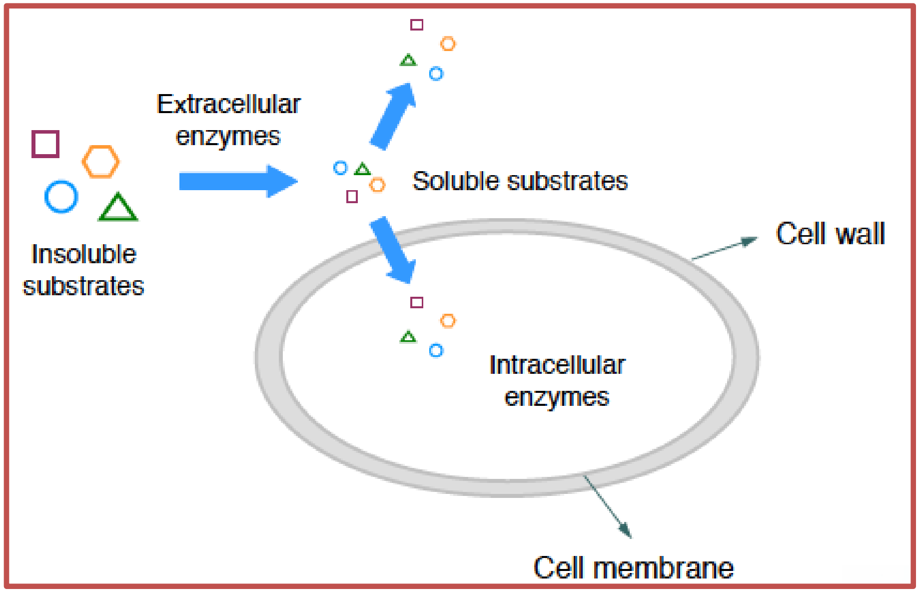
3 Acetogenic becteria

4 Methanogenic archea

5 Sulphate reducing bacteria

Enzymes used in AD

Stage of AD	Enzymes
Hydrolysis: Solubilization of particulate and colloidal wastes	Exoenzymes
Acid forming: Conversion of soluble organic acids and alcohols to acetate, carbon dioxide, and hydrogen	Endoenzymes
Methanogenesis: Production of methane and carbon dioxide	Endoenzymes



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Hydrolytic organisms

- Act by secreting extra cellular enzymes which break the bonds of polymeric substances producing shorter chain compounds

- Attach the surface of the substrate using a secreted 'sticky' extracellular polymeric substances (EPS)

- Fast growth rates but hydrolysis can be rate limiting in highly cellulosic substances

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Hydrolytic organisms

- The fastest stage in anaerobic systems
- Rarely a rate limiting step
- Biological reaction can be:
  - Intracellular, performed by intracellular enzymes
  - Extracellular, performed by extracellular enzymes

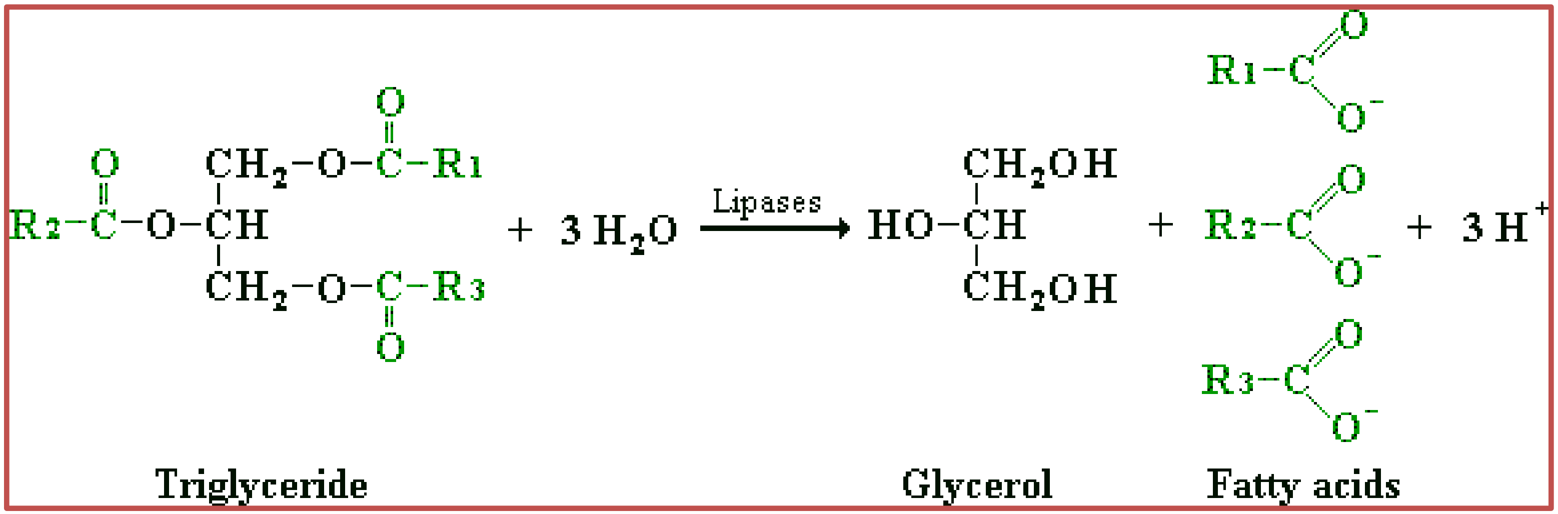
Substrate to be Degraded	Exoenzyme Needed	Example	Bacterium	Product
Polysaccharides	Saccharolytic	Cellulase	*Cellulomonas*	Simple sugar
Proteins	Proteolytic	Protease	*Bacillus*	Amino acids
Lipids	Lipolytic	Lipase	*Mycobacterium*	Fatty acids

REACTIONS

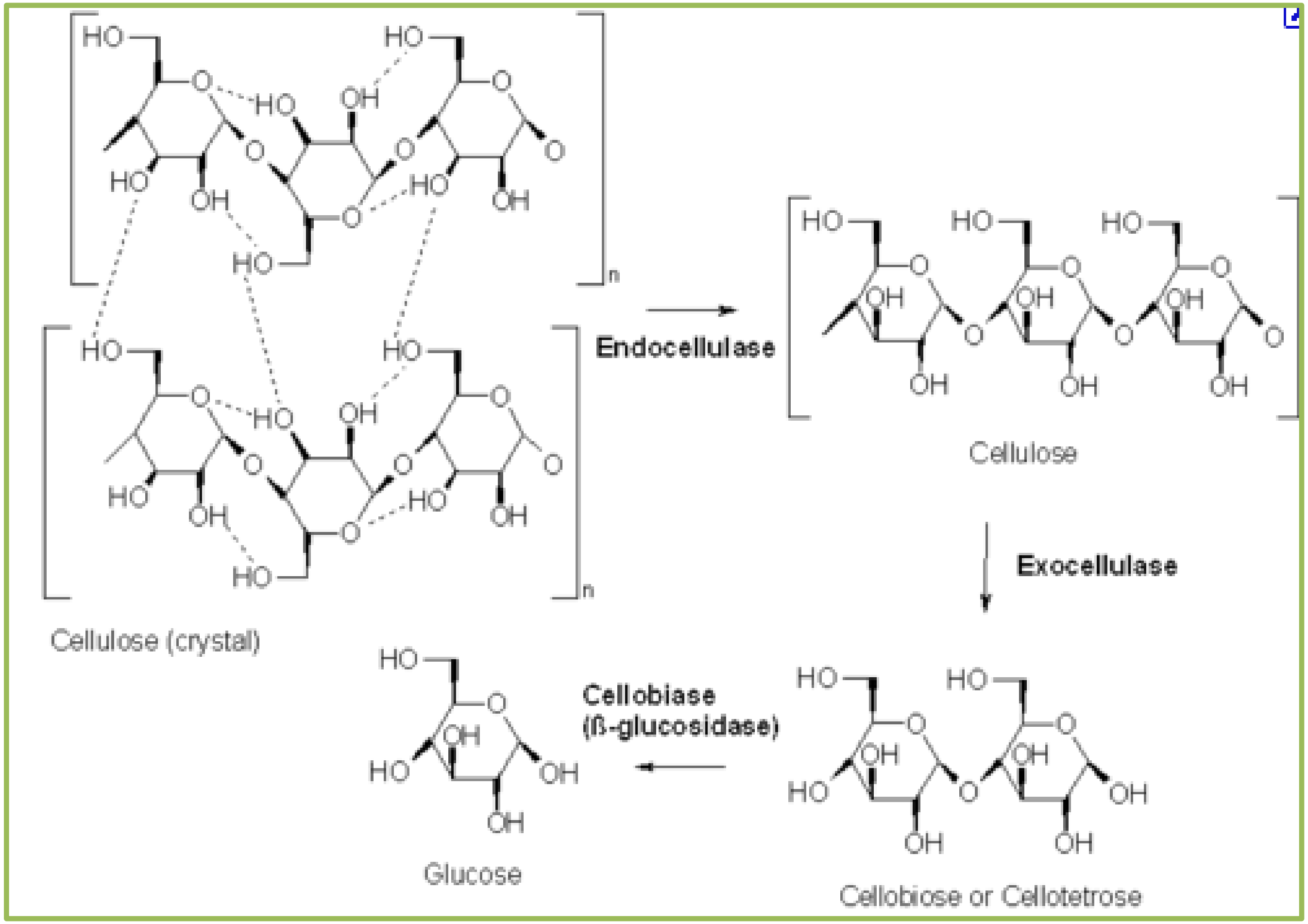
-Carbohydrates	→	sugars, alchools
-Cellulose	→	glucose, cellobiose
-Lignin	→	degraded very slowly
-Proteins	→	Aminoacids, peptides
-Fats	→	Fatty acids, glycerol

Hydrolytic enzyme

- Example: Fat hydrolysis by lipase



Hydrolysis of cellulose



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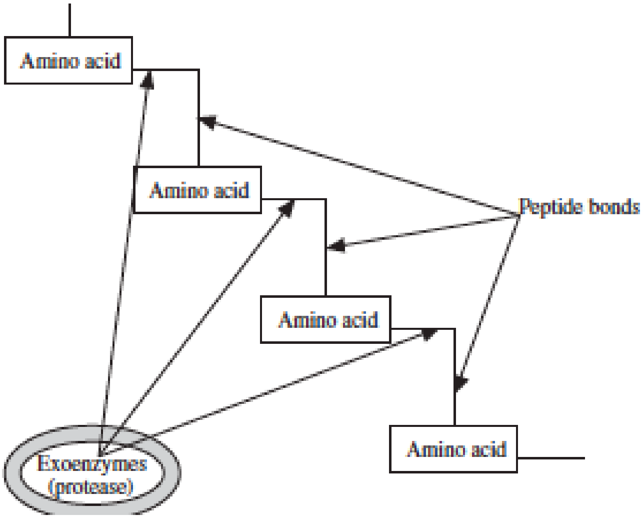
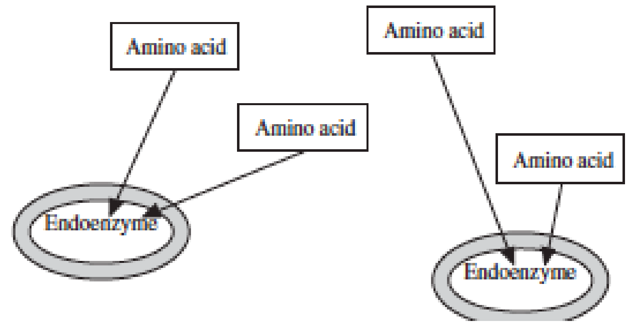
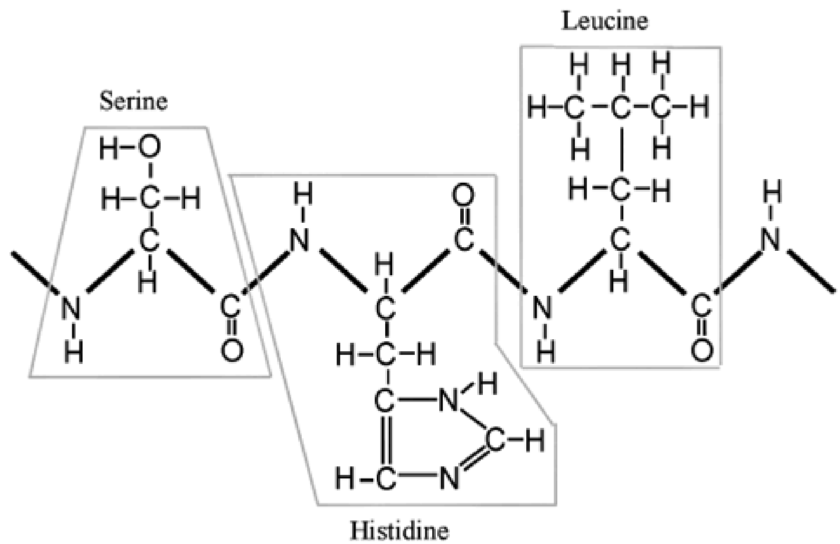
Hydrolysis of proteins







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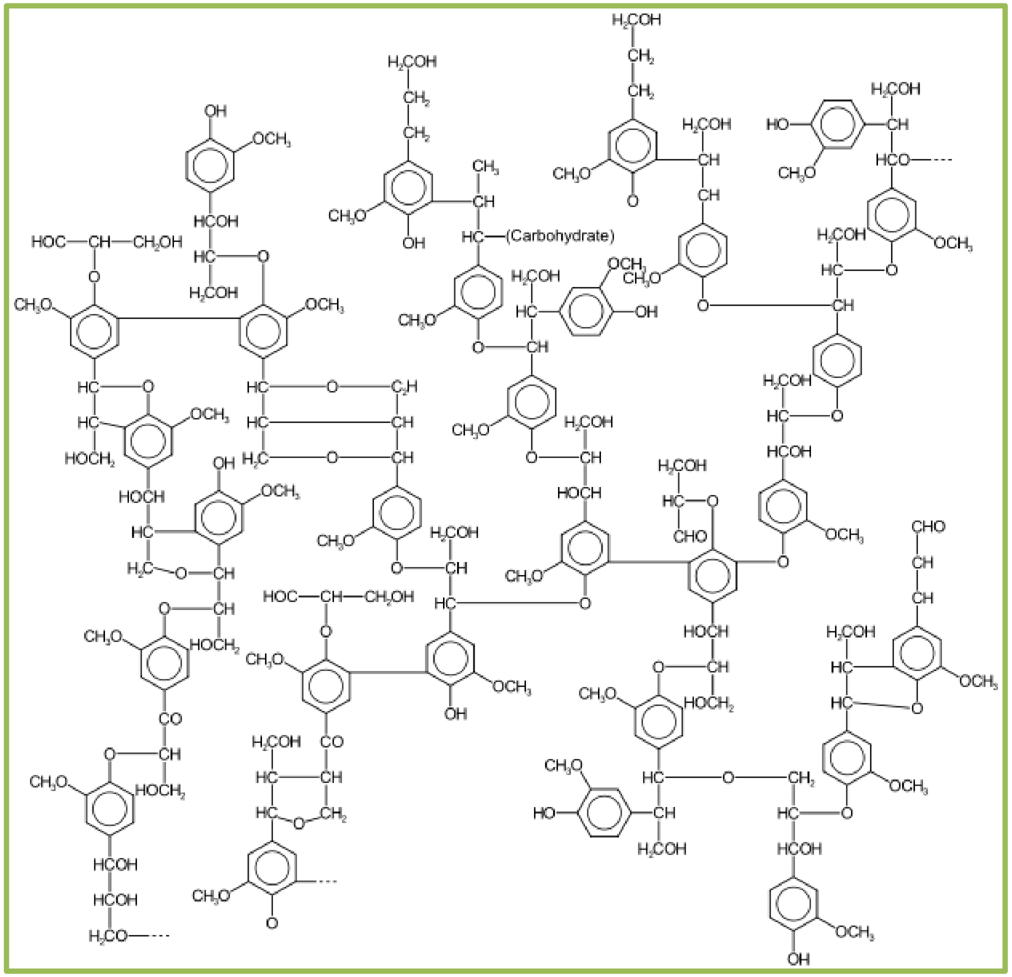
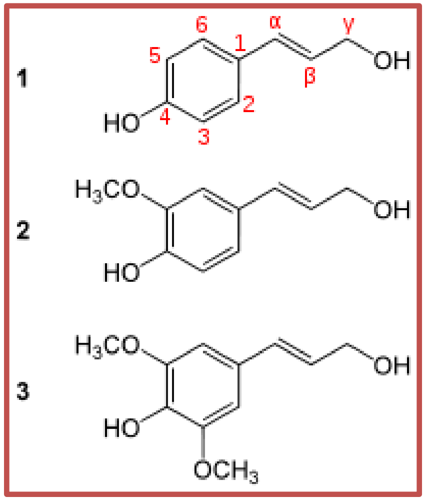
Lignine



The three common monolignols: paracoumaryl alcohol (1), coniferyl alcohol (2) and sinapyl alcohol (3)



Fermentative bacteria (acidogenic microorganisms)

REACTIONS:

- Sugars → Fatty Acids (succinate, acetate, proprionate, lactate, formate), carbon dioxide, hydrogen
- Amino Acids → Fatty Acids, ammonia, sulphides, carbon dioxide, hydrogen
- Glycerol → acetate, cabon dioxide
- Alchools → Fatty Acids, carbon dioxide

Fermentative bacteria (acidogenic microorganisms)

Fermentative Pathway	Products	Representative Bacterial Genus
Acetone–butanol	Acetone, butanol, ethanol,	*Clostridium*
Butanediol	Acetate,2,3-butanediol, butylene, ethanol, gylcol, lactate, CO_2, H_2	*Enterobacter*
Butyrate	Acetate, butyrate, CO_2, H_2	*Clostridium*
Lactate	Lactate	*Lactobacillus*
Mixed acid	Acetate, ethanol, lactate, CO_2, H_2	*Escherichia*
Propionate	Propionate	*Propionibacterium*

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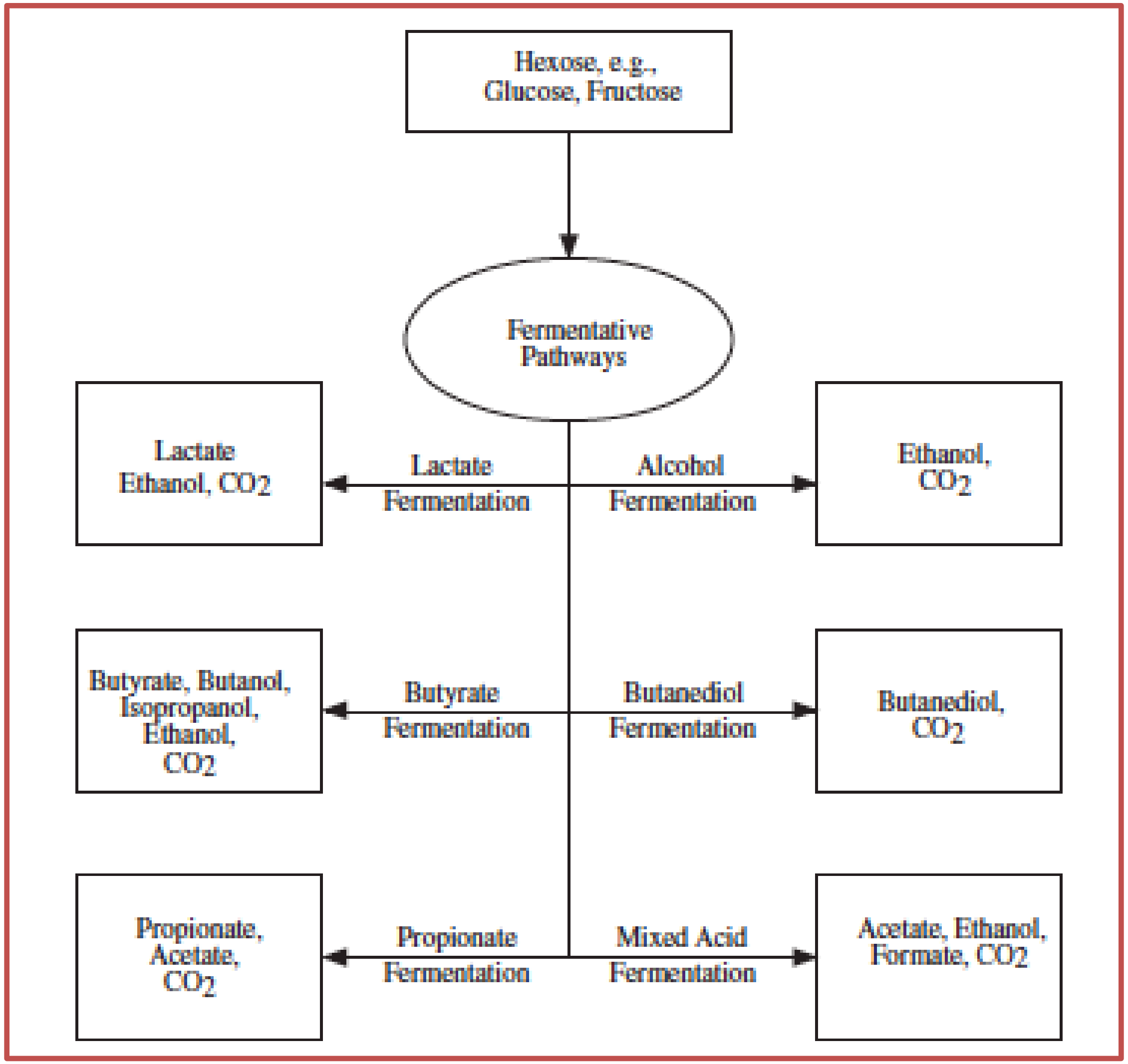
Type of fermentation



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Acetogenic microorganism

-Acetogenic bacteria produce acetic acid, hydrogen and carbon dioxide from fermentation products

-Fall into two main groups:

- Hydrogen producing acetogens
- Homoacetogens

- Slow growth rates
- Sensitive to physical and chemical conditions (temperature, pH, hydrogen partial pressure)
- Work in synergy with methanogenic microorganisms-interspecies hydrogen transfer

Hydrogen producing acetogens

-Butyrate

$CH_3CH_2CH_2COOH + 4H_2O \rightarrow CH_3COOH + 2CO_2 + 6H_2$

-Proprionate

$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$

-Propanol

$CH_3CH_2CH_2OH + 3H_2O \rightarrow CH_3COOH + CO_2 + 5H_2$

Homoacetogens

$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$

Methanogens

- Present in natural habitats (sediments, digestive systems) and are responsible for the production of methane from a wide variety of methylated compounds

- Slow growth rates

- Sensitive to physical conditions (pH, temperature) and can be inhibited by many compounds

- Main methanogens in anaerobic digesters fall into two main groups

  - Acetoclastic-acetate degrading
  - Hydrogenotrophic-hydrogen utilising

Methanogens

Acetoclastic methanogens *Methanosarcina, Mathanosaeta (Methanothrix)*

$$CH_3COOH \rightarrow CH_4 + CO_2$$

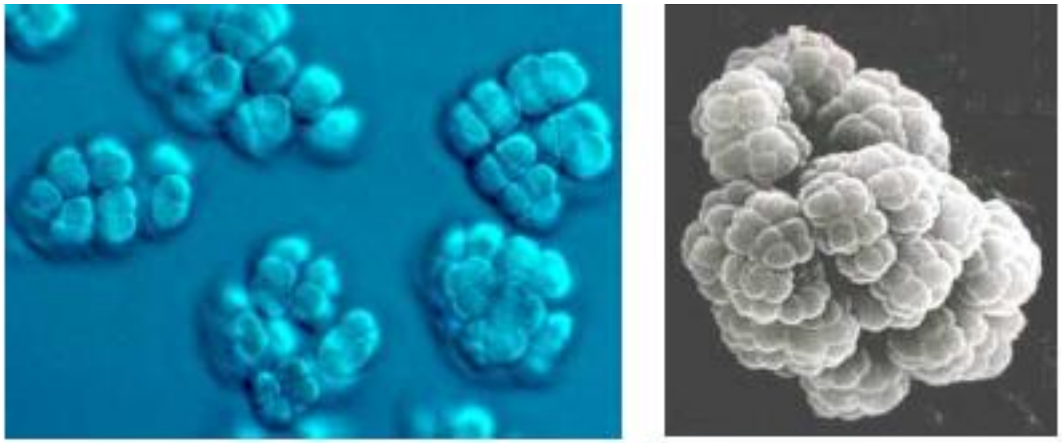
- 2/3 of methane produced by this route
- Slowest growth rate and most sensitive organisms

Hydrogenotrophic methanogens

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

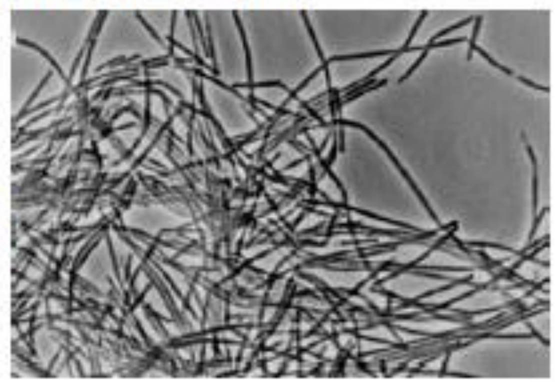
- 1/3 of methane produced by this route
- Higher growth rate and less sensitive organisms

Acetoclastic methanogens



Methanosarcina acetivorans

Methanosaeta concilii



Methanosaeta sp.

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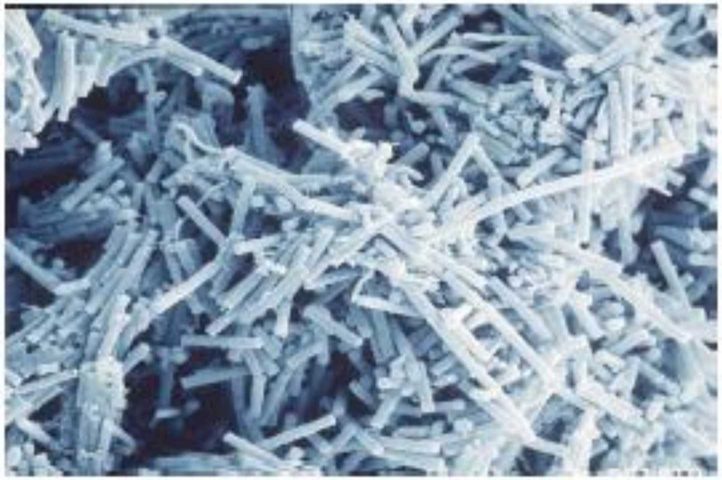
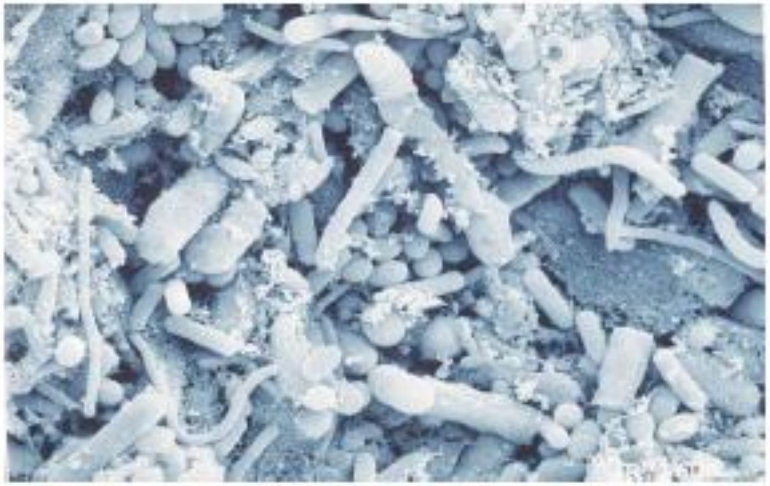
Methanogens



Methanosaeta
Substrate: Acetate



Mixed culture
Substrate: Sucrose

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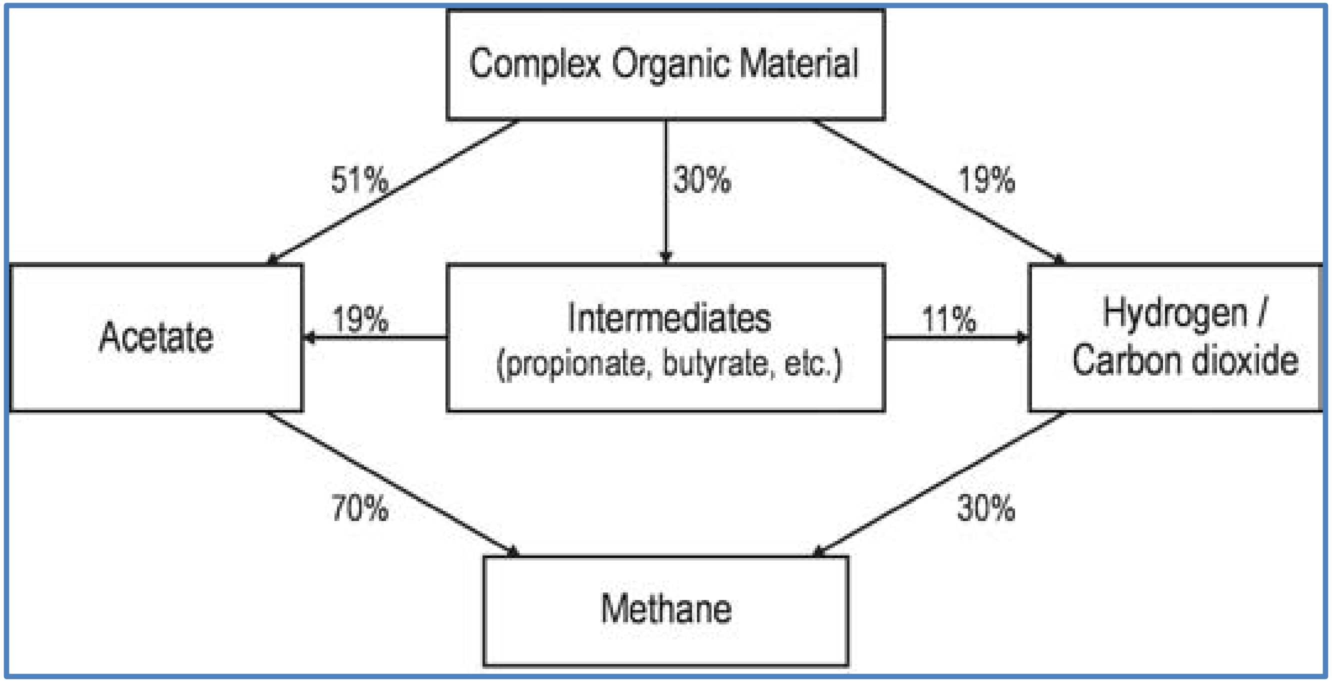
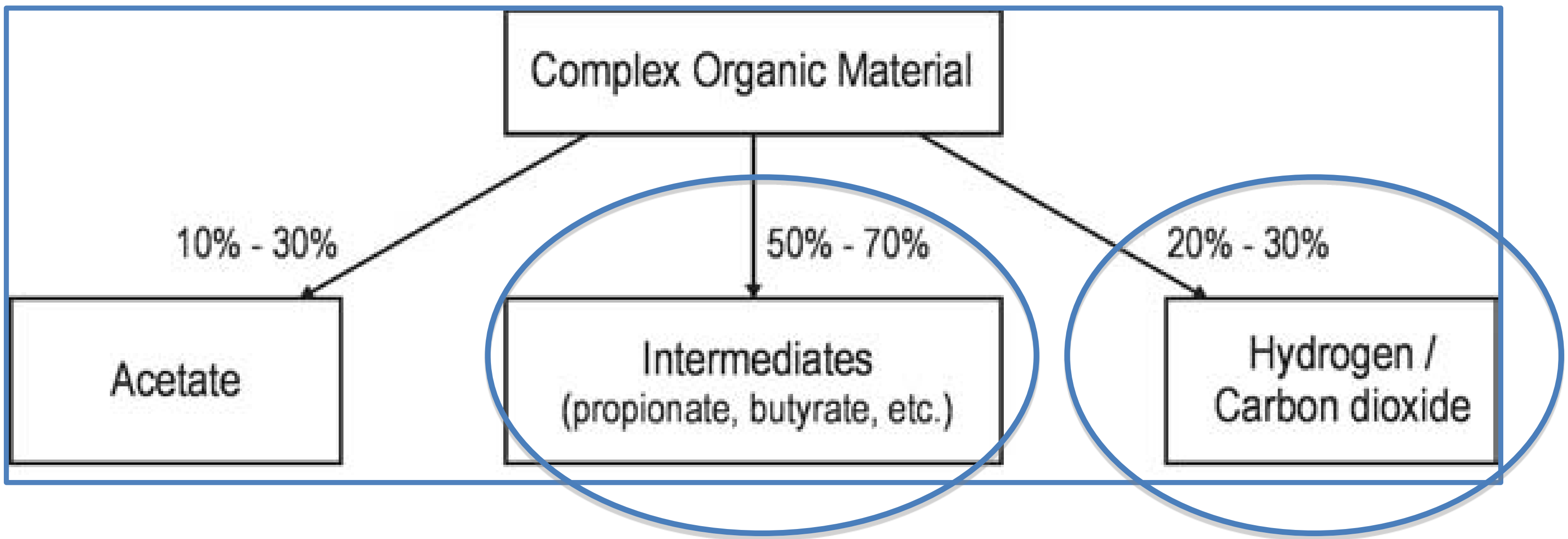
Carbon flow in anaerobic environments with active methanogens



Only between 20 and 30% of the carbon is transformed into intermediary products before these are metabolized to methane and carbon dioxide

Carbon flow in anaerobic environments without active methanogens



(basic mechanism of a two phase approach to produce VFA and Hydrogen)

FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM:

The rate and efficiency of the anaerobic digestion process is controlled by:

· The type of waste being digested,
· Process temperature,
· The presence of toxic materials,
· The pH and alkalinity,
· The hydraulic retention time,
· The rate of digester loading,

FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM:

- Competition between different organisms for the same substrate
- Synergies between different groups of organisms
- Physical/Chemicals factors:
  - feedstock composition
  - feedstock structure
  - pH
  - pH equilibriums
  - nutrients (trace elements)
  - inhibition (VFA, ammonia)
  - liquid/gas transfer etc. (H_2 partial pressure)
- Chemical reactions are catalyzed by biological enzymes (biochemical process).

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FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **pH**

Each group of organisms have an pH optima for maximum rate of reaction

- methanogens: pH7-8 optimal, pH 6.5-8.5 operational
- fermentation: pH 5-7 optimal
- hydrolysis: pH 5-7 optimal

Optimal pH gives higest rate related

Deviation from optimum value could be:

-Introduced with the influent
-Consequenced by excess production and accumulation of acidic or basic conversion products such as VFA or Ammonia.

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FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: pH

Equilibria

Ionic equilibriums can have a large effect on the AD process since undissociated (non-ionic) forms can pas through cell membranes and cause inhibition

Organic substrate $+H_2O$ →

CH_4 ↑

\+

CO_2 ↑ $<-> H_2CO_3 <-> H^+ + HCO_3^- <-> H^+ + CO_3^{--}$

\+

$NH_3 <-> NH_4^+ + OH^-$

\+

$R\ COOH <-> H^+ + RCOO^-$

\+

$H_2S <-> H^+ + HS^- <-> H^+ + S^{--}$

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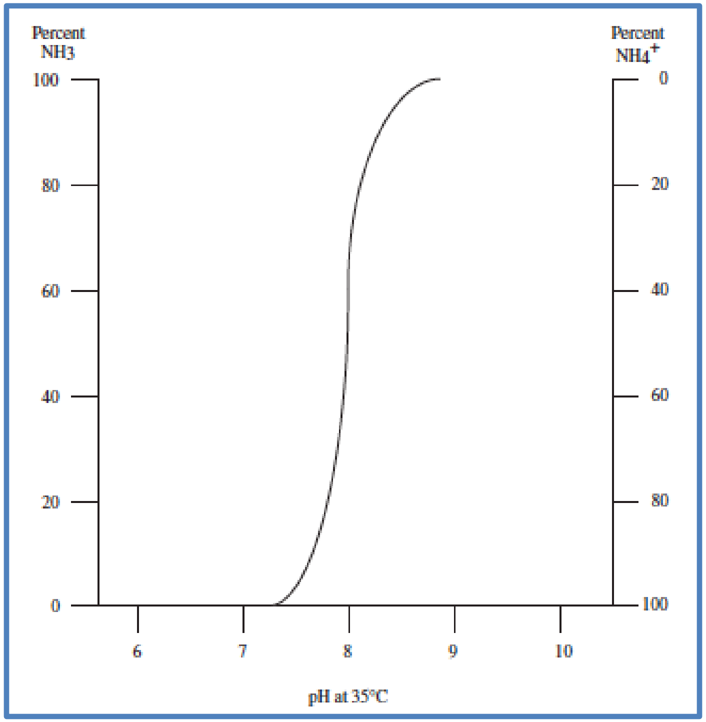
FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: pH

Eg Ammonia $NH_3 + H_2O \leftarrow\rightarrow NH_4^+ + OH^-$

Free ammonia (non ionic) is more inhibitive to the AD process that the ammonium ion so for a given ammonia-nitrogen concentration lower pH is favored by the process.



FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **pH**

Acidic ← → Alkaline Relationship

Organic substrate

Fatty acid
CH_3COOH <-> $CH_3COO^- + H^+$
pKa= 4.76

CO_2, H_2O, NH_3, H_2S

H_2CO_3 <-> $NH_4^+ + HCO_3^-$
Bicarbonate alkalinity

Acetic acid and hydrogen sulphide are both more inhibitive at lower pH since the non ionic forms is prevalent.

FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **pH**

Acetic acid could be inhibitive at lower pH since the non ionic forms is prevalent.

$CH_3COOH \leftrightarrow CH_3COO^- + H^+$
pKa= 4.76

Non ionic form of acetate is able to pass through the membrane. At low pH (<5) the non ionic form is prevalent. This could cause an overload of acetic acid inside the cell.

At higher pH value (>8) acetic acid is in his ionic form, and it is unable to pass the membrane causing an accumulation outside the cell.

FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **NITROGEN**

- Is mainly present in protein (also Urea)
- In the AD nitrogen is converted to ammonia
- Ammonia is known to be beneficial at low concentrations (about 200 mg/l) but can be inhibitive at high concentration:

  - complex inhibition mechanism
  - ammonia is antagonistic/synergistic with other substances

- Anaerobic digesters can become acclimatized to high ammonia concentrations:

  - due to a shift in the internal mechanism of methanogens?
  - a shift in the dominant species in the digester?

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FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **HYDROGEN**

- Hydrogen producers: acidogens and acetogens, thermodynamics unfavourable at high hydrogen concentration.
- Hydrogen consumers: hydrogenotrophic methanogens and homoacetogens, require hydrogen as substrate to produce methane and acetate.
- Hydrogen plays an important intermediary role during acetogenesis, as the reaction will only occur if the hydrogen partial pressure is low enough to thermodynamically allow the conversion of all the acids. Such lowering of the partial pressure is carried out by hydrogen scavenging bacteria, thus the hydrogen concentration of a digester is an indicator of its health.

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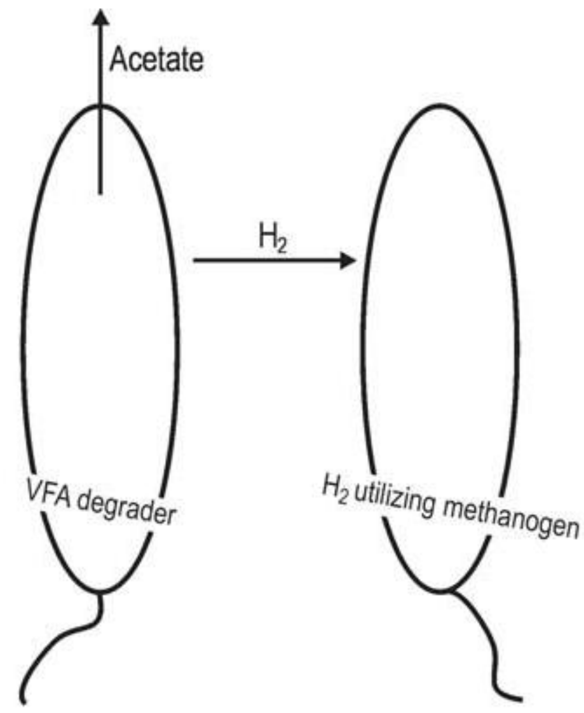
FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **HYDROGEN**

- Interspecies hydrogen transfer: a symbiotic relationship between methanogenic and acetogenic/acidogenic anaerobic microorganisms
- Hydrogen consumers are constantly supplied with substrate
- Hydrogen producers have hydrogen removed from solution allowing them to continue to metabolize their substrates



FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **SULPHUR**

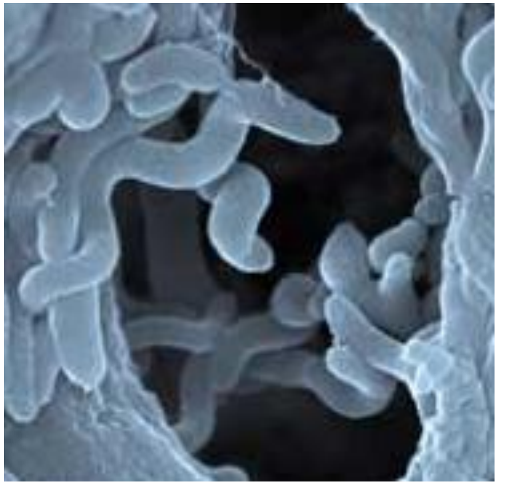
- Sulphur is present in all biological materials especially those containing high concentration of protein.

- Sulphates present in the feed material are reduced to hydrogen sulphide by sulphide reducing bacteria (SRB)

- SRB compete with methanogenic organisms for the same substrates in order to reduce sulphur:

- $H_2 + SO_4^{2-} \rightarrow H_2S + H_2O$

- $CH_3COOH + SO_4^{2-} \rightarrow H_2S + CO_2 + H_2O$

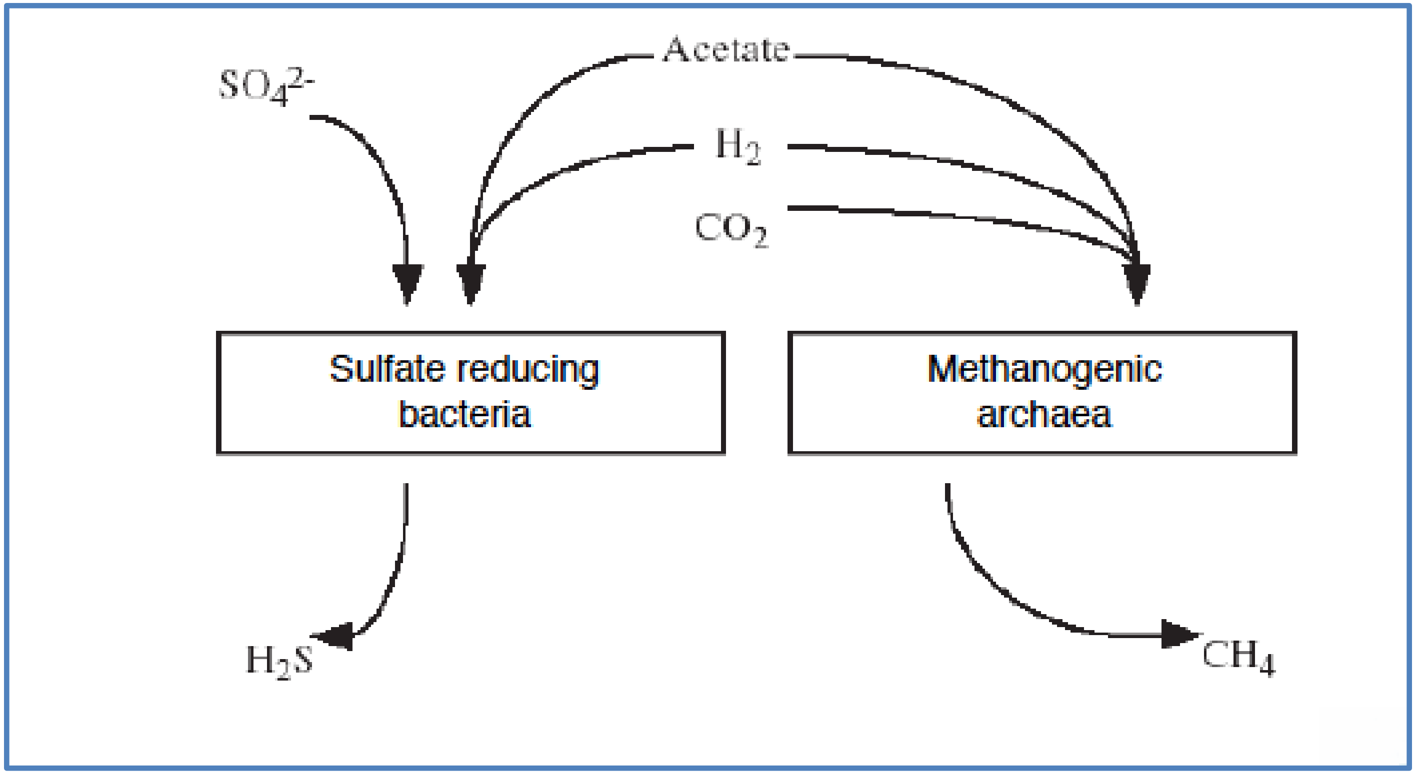


- SRB reduce the total biogas production from AD

- Sulphid is toxic to many organisms (200-1500 mg/l)

FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **SULPHUR**

Competition between SRB and methanogens



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FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: SULPHUR

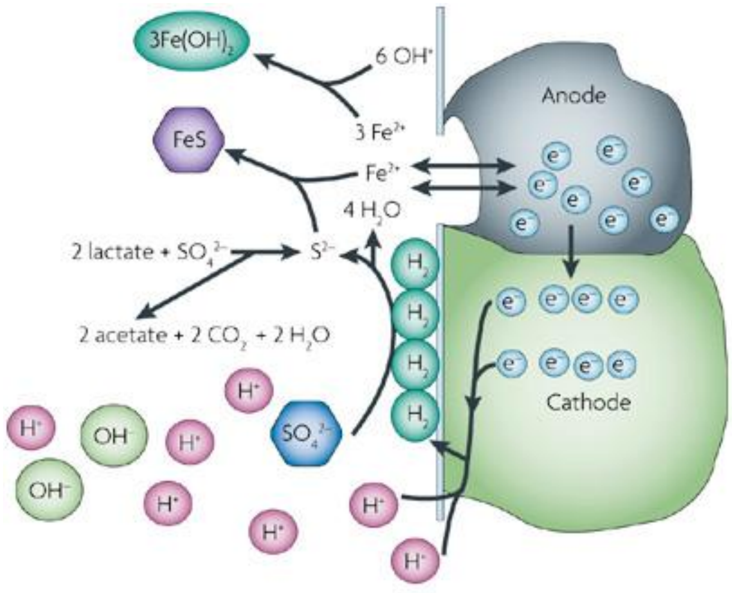
- The presence of sulphates causes numerous disadvantages to the commercial anaerobic digestion process

- Quality of biogas is lower due to hydrogen sulphide (H_2S) content:

  - hydrogen sulphide (H_2S) has a strong unpleasant odour
  - hydrogen sulphide is corrosive to machinery

- Biogas must me cleaned before use

- Metals can be precipitated from digestate as sulphides (leading to nutrient deficiencies) causing failure of the process



FACTORS THAT CAN AFFECT THIS COMPLEX SYSTEM: **NUTRIENT**

- AD has low nutrient requirements
- Nutrient are often inhibitive in high concentrations
- Usually feedstock is nutrient sufficient
- In nutrient limited systems supplementation can have a positive impact
- Nutrient limitations can be caused by precipitation of sulphide.

Element	Enzyme	Anaerobic microorganism
Selenium	Formate hydrognase	Acetogenic bacteria
	Glycine reductase	Several clostridia
	Hydrogenase	Methanococcus vanielii
	Nicotin acid hydroxylase	Clostridium barkeri
	Xanthine dehydrogenase	Some clostridia
Tungsten	Formate dehydrogenase	Acetogenic bacteria
Nickel	Carbon monoxide dehydrogenase	Some clostridia
	hydrogenase	Methanobacterium, desulfovibrio gigas
	Methyl reductase	Methanogenic bacteria

BASIC CONCEPT TO TAKE HOME……..

When biogas yields of your AD reactor in not so satisfactory,

Take care of who is working together to produce it and try to understand what is the inhibition factor that make them