ENVE 424
Anaerobic Treatment

Lecture 3
The Microbiology of Anaerobic Treatment

2012 – 2013 Fall

27 - 28 Sept 2012

Assist. Prof. A. Evren Tugtas

Marmara Üniversitesi

Anaerobic Digestion

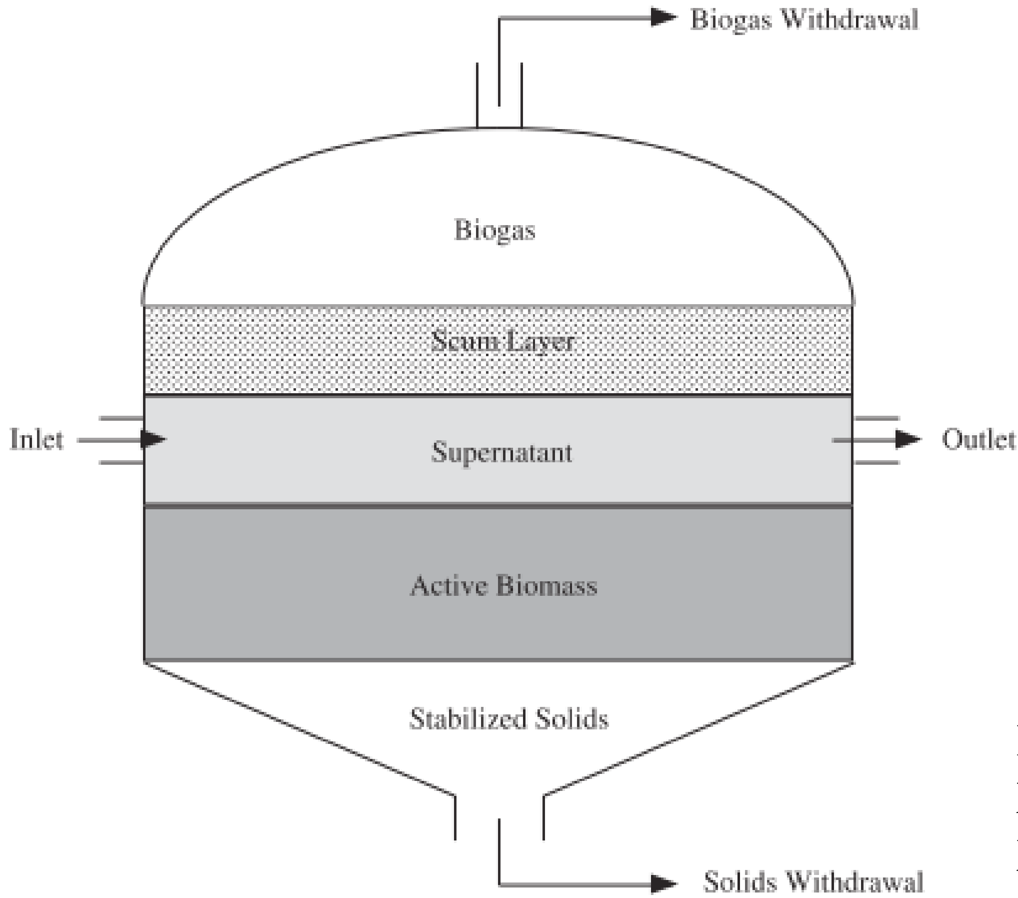


Figure 1.1 *Suspended growth anaerobic digesters are commonly used at municipal wastewater treatment plants for the degradation of primary and secondary sludges. These digesters produce several layers as a result of sludge degradation. These layers are from top to bottom: biogas, scum, supernatant, active biomass or sludge, and stabilized solids.*

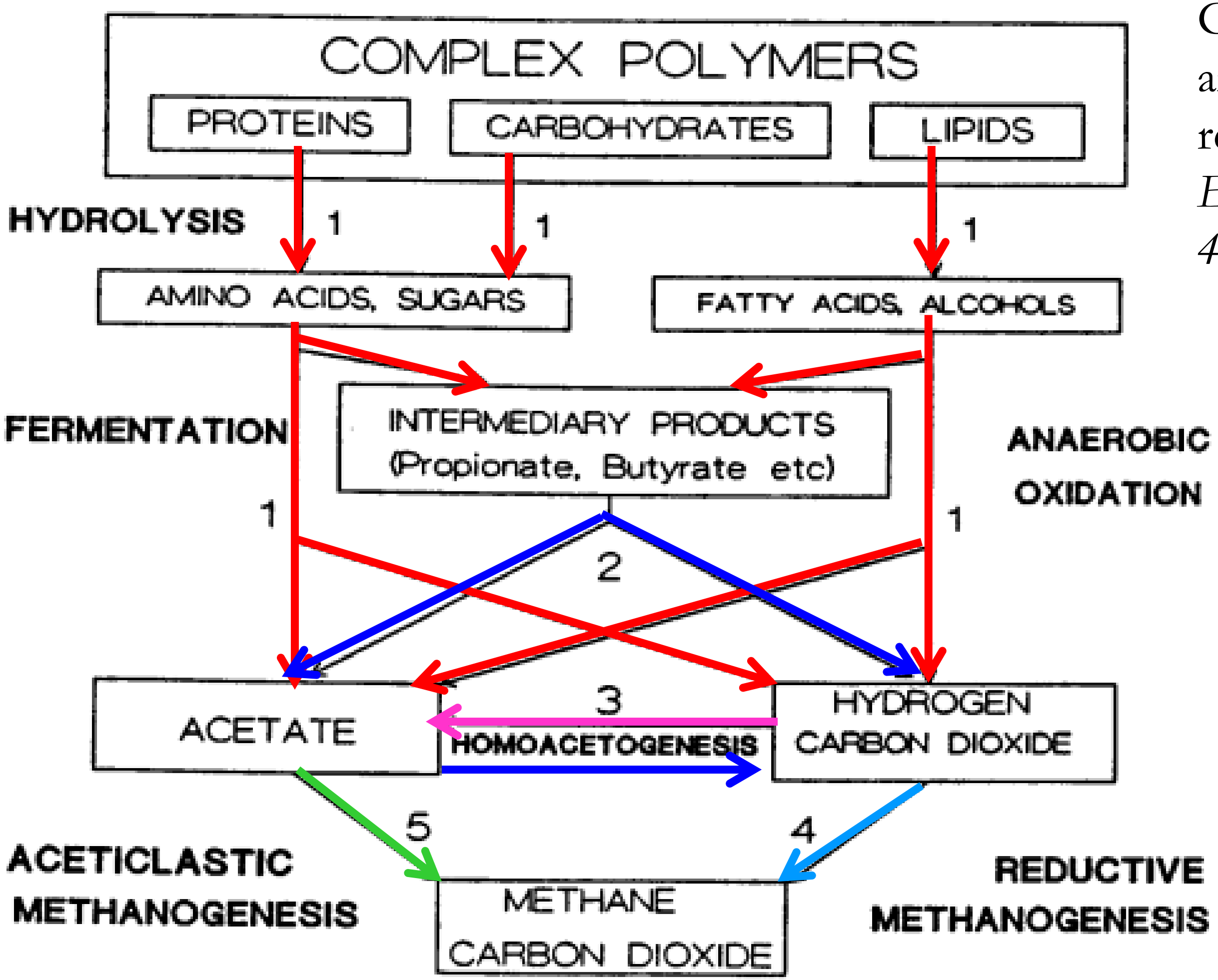
Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Process Microbiology

The consortia of microorganisms involved in the overall conversion of organic matter to methane

- **Bacteria** hydrolyze complex organic matter to simple carbohydrates, aminoacids, fatty acids.
- Simple carbohydrates and acids utilized by fermenting **bacteria** for energy and growth, producing organic acids and H_2.
- Organic acids are partially oxidized by other fermentative **bacteria**, which produce additional hydrogen and acetic acid
- Hydrogen and acetic acid are used by **archael methanoges**, which convert them into methane

Microbiology of 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.*

Numbers indicate bacterial groups

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

Marmara Üniversitesi

Process Microbiology

- Microorganisms are divided into three groups according to their response to molecular oxygen

1) Strict aerobes
2) Facultative anaerobes
3) Anaerobes (methanogens)

Response to free molecular O_2

TABLE 2.1 Groups of Bacteria According to Their Response to Free Molecular Oxygen

Group	Example	Significance
Strict aerobes	*Haliscomenobacter hydrossis*	Degrades soluble organic compounds; contributes to filamentous sludge bulking
	Nitrobacter sp.	Oxidizes NO_2^- to NO_3^-
	Nitrosomonas sp.	Oxidizes NH_4^+ to NO_2^-
	Sphaerotilus natans	Degrades soluble organic compounds; contributes to filamentous sludge bulking
	Zoogloea ramigera	Degrades soluble organic compounds; contributes to floc formation
Facultative anaerobes	*Escherichia coli*	Degrades soluble organic compounds; contributes to floc formation; contributes to denitrification or clumping
	Bacillus sp.	Degrades soluble organic compounds; contributes to denitrification or clumping
Anaerobes	*Desulfovibrio* sp.	Reduces SO_4^{2-} to H_2S
	Methanobacterium formicium	Produces CH_4

Marmara Üniversitesi

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Process Microbiology

TABLE 2.2 Groups of Anaerobic Bacteria

Group	Example	Significance
Oxygen tolerant	*Desulfovibrio* sp.	Reduces SO_4^{2-} to H_2S
	Desulfomarculum sp.	Reduces SO_4^{2-} to H_2S
Oxygen intolerant	*Methanobacterium formicium*	Produces CH_4
	Methanobacterium propionicium	Produces CH_4

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Electron transport

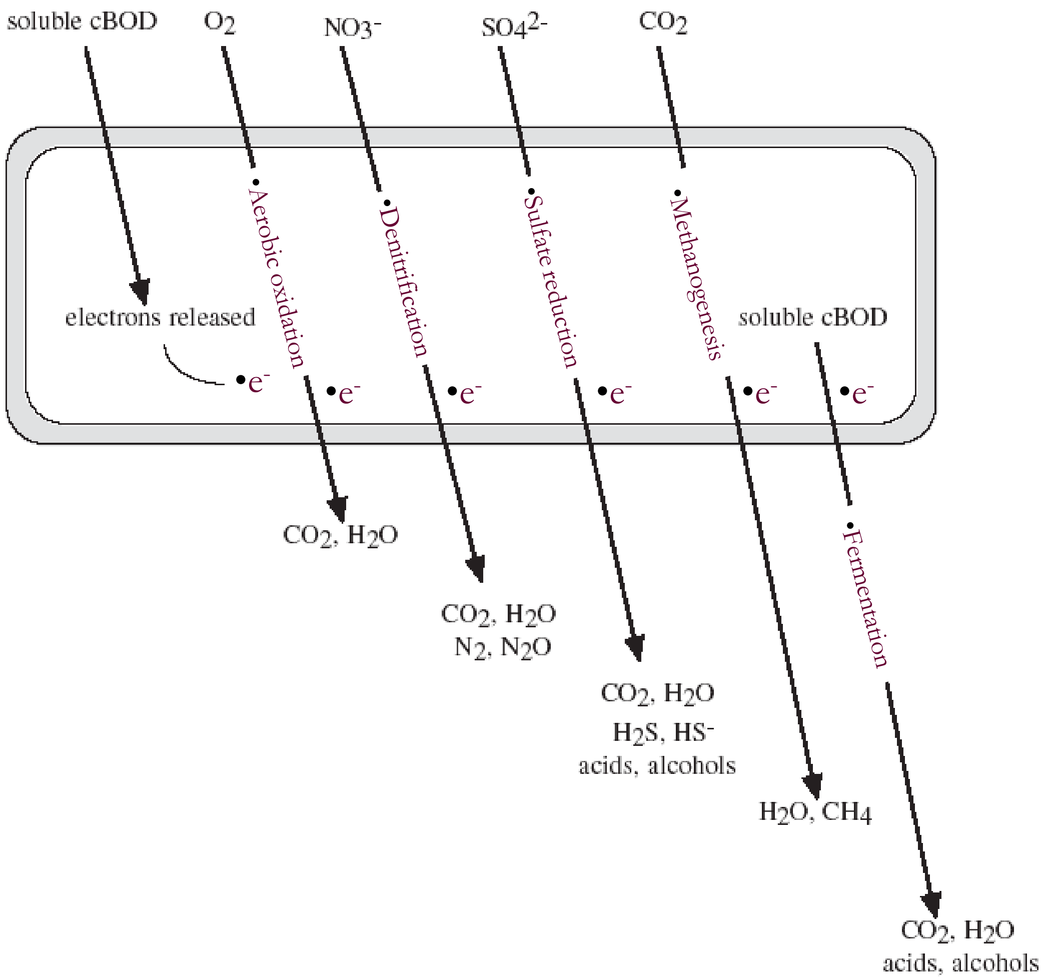
Operational Condition	Transport Molecule	Biological Process
Aerobic	O_2	Aerobic degradation of substrate
Anoxic	NO_3^-, NO_2^-	Anoxic degradation of substrate
Anaerobic	SO_4^{2-}	Sulfate reduction and degradation of substrate
Anaerobic	CO_2	Methanogenesis
Anaerobic	Organic molecule	Fermentation and degradation of substrate

- Electron transport molecules (e^- acceptors) used in degradation of soluble substrate

Marmara Üniversitesi

Electron transport

Depending upon the molecule used (O_2, NO_3^-, SO_4^{2-}, CO_2, or soluble cBOD) by bacteria to remove electrons from the cell, a variety of substrates are produced.



Oxidation-Reduction Potential vs Cellular activity

Approximate ORP, mV	Final Electron Acceptor (carrier)	Condition	Respiration
>+50	O_2	Oxic	Aerobic
+50 to –50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<–50	SO_4^{2-}	Anaerobic	Fermentation, sulfate reduction
<–100	Organic Compound	Anaerobic	Fermentation, mixed acid production
<–300	CO_2	Anaerobic	Fermentation, methane production

Marmara Üniversitesi

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Energy yield and sludge production

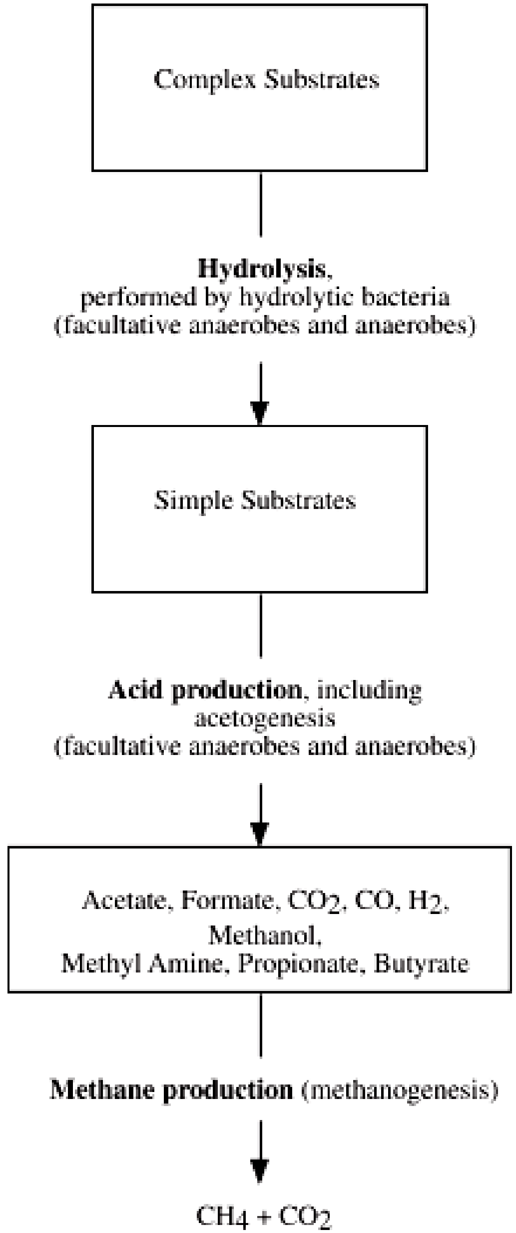
Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Final Electron Carrier Molecule	Form of Respiration	Energy Yield Rank	kg VSS Produced per kg COD Degraded
O_2	Aerobic or oxic	1	~0.4–0.6
NO_3^-	Anaerobic or anoxic	2	~0.4
SO_4^{2-}	Anaerobic: sulfate reduction	3	0.04–0.1
Organic molecule	Anaerobic: mixed acids and alcohol	4	0.04–0.1
CO_2	Anaerobic: methane production	5	0.02–0.04

Bacterial Group	Yield (kg VSS/kg COD)
Volatile acid-forming bacteria	0.15
Methane producing archaea	0.03

Marmara Üniversitesi

Three steps of Anaerobic Digestion



Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Three steps of Anaerobic Digestion

Hydrolysis

Complex carbohydrates -----> Simple sugars
Complex lipids -----> Fatty acids
Complex proteins -----> Amino acids

Acid Production

Simple sugars + fatty acids + amino acids -----> organic acids, including acetate + alcohols

Acetogenesis (acetate production)

Organic acids + alcohols -----> acetate

Methane production: acetoclastic methanogenesis

Acetate → $CH_4 + CO_2$

Methane production: hydogenotrophic methanogenesis

$H_2 + CO_2$ → CH_4

Methane production: methyltrophic methanogenesis

Methanol → $CH_4 + H_2O$

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Extracellular (exo)enzymes

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

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

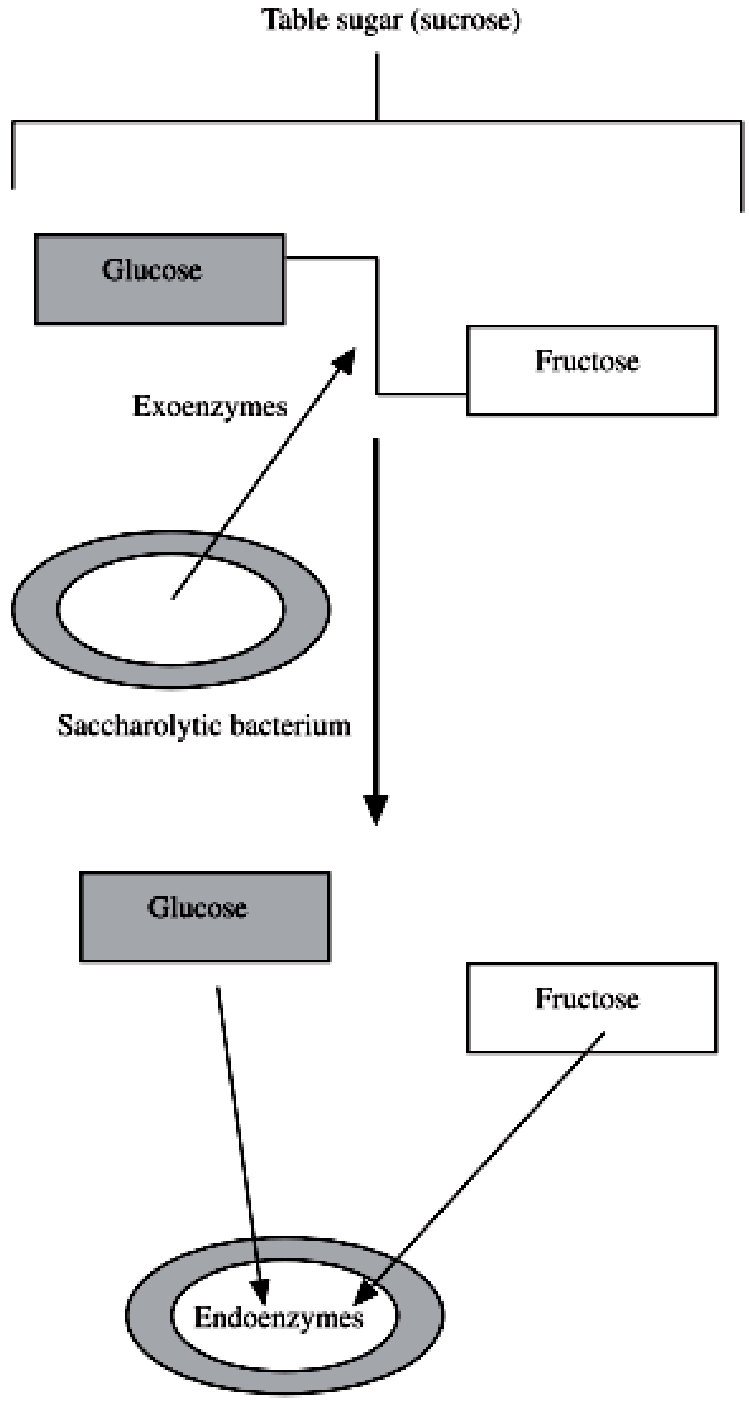
Marmara Üniversitesi

Enzymes used in AD

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

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

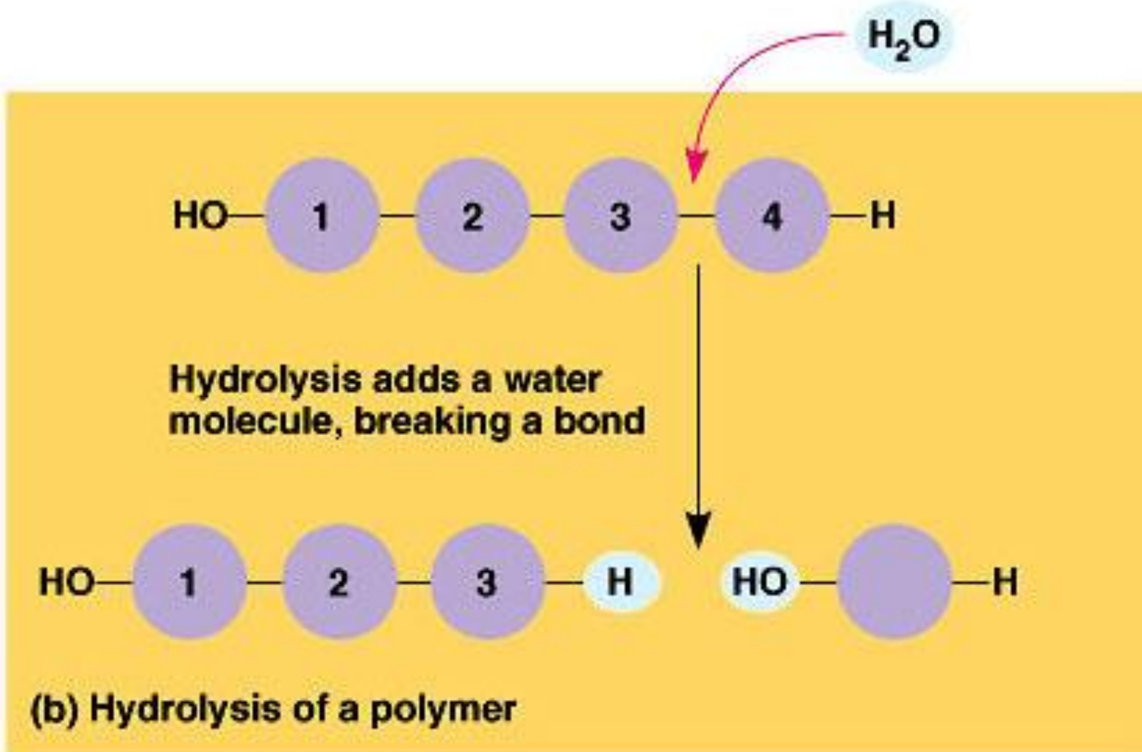
Enzymes



Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Marmara Üniversitesi

Step 1 - Hydrolysis



HYDROLYSIS...

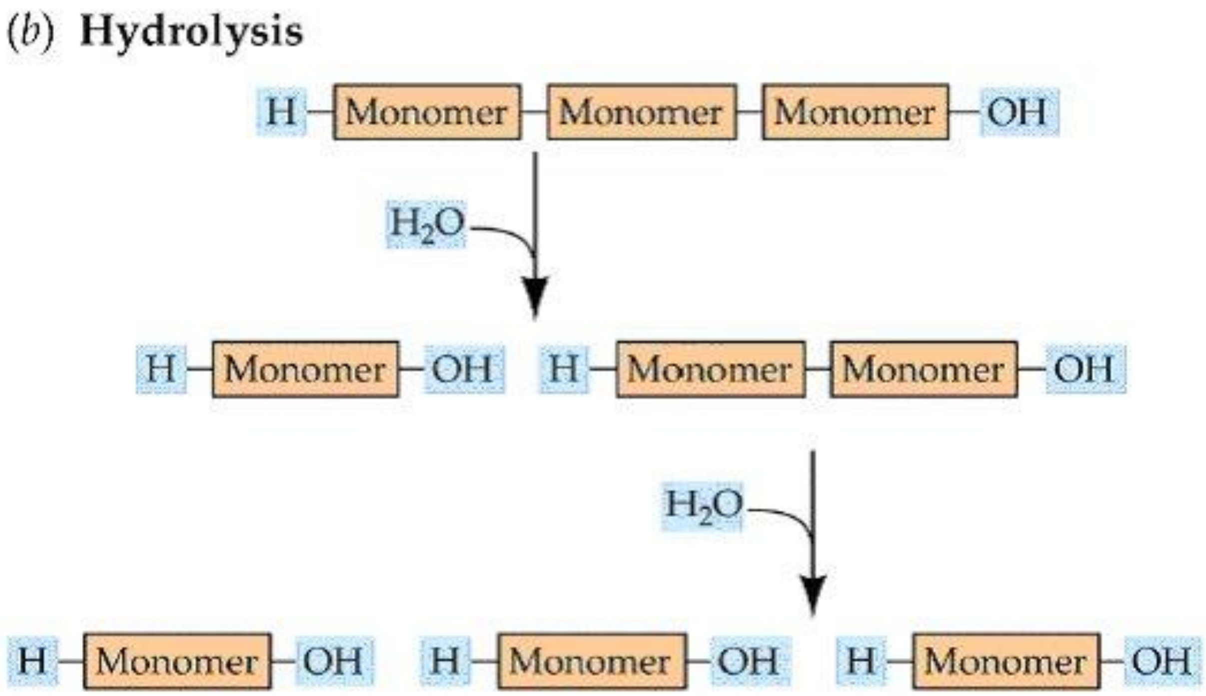
the splitting of a polymer by adding water to a covalent bond;

catalyzed by a hydrolyase enzyme.

Ref: http://bealbio.wikispaces.com/Period+3+ch+5+Q

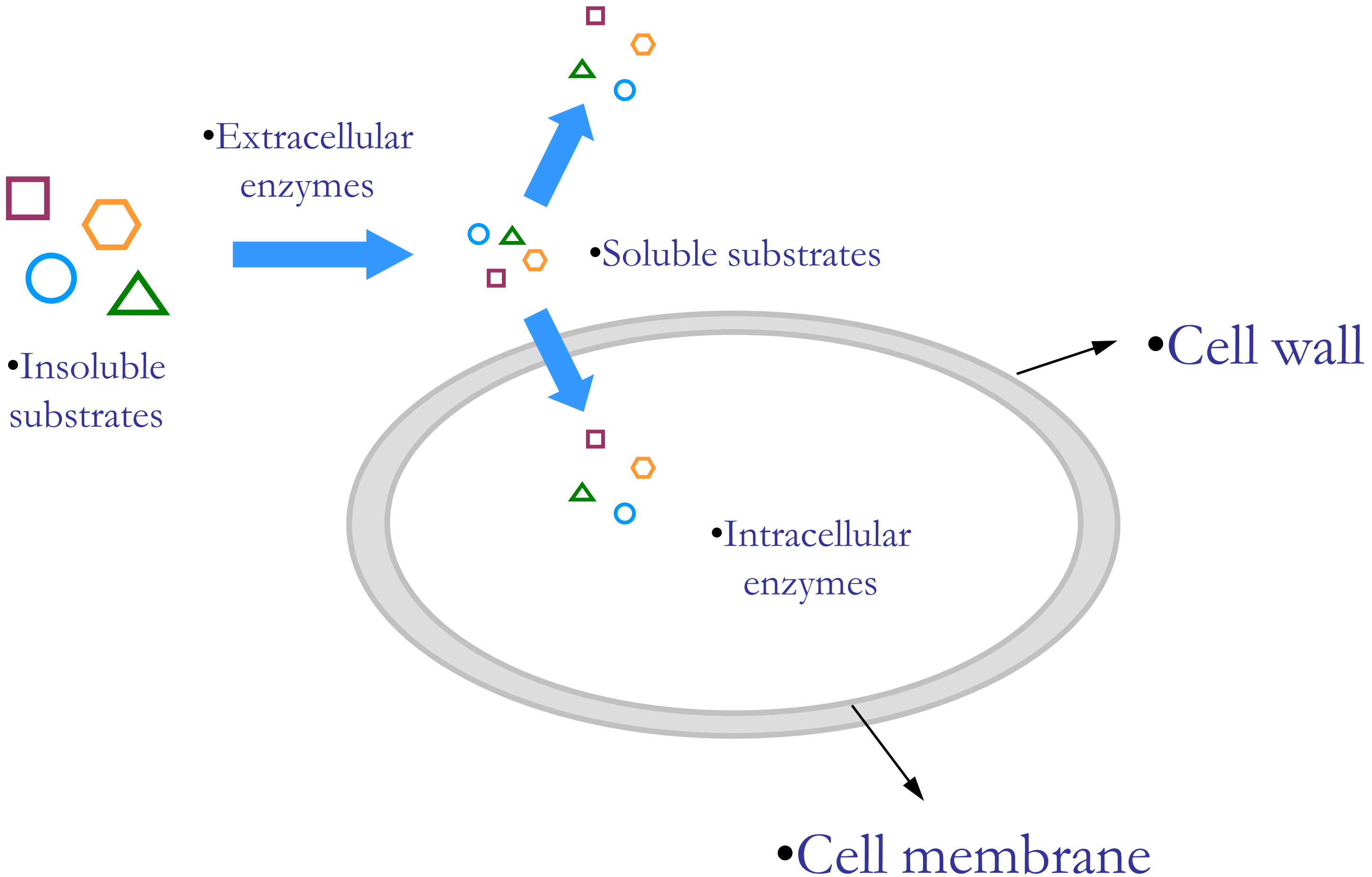
Marmara Üniversitesi

Hydrolysis

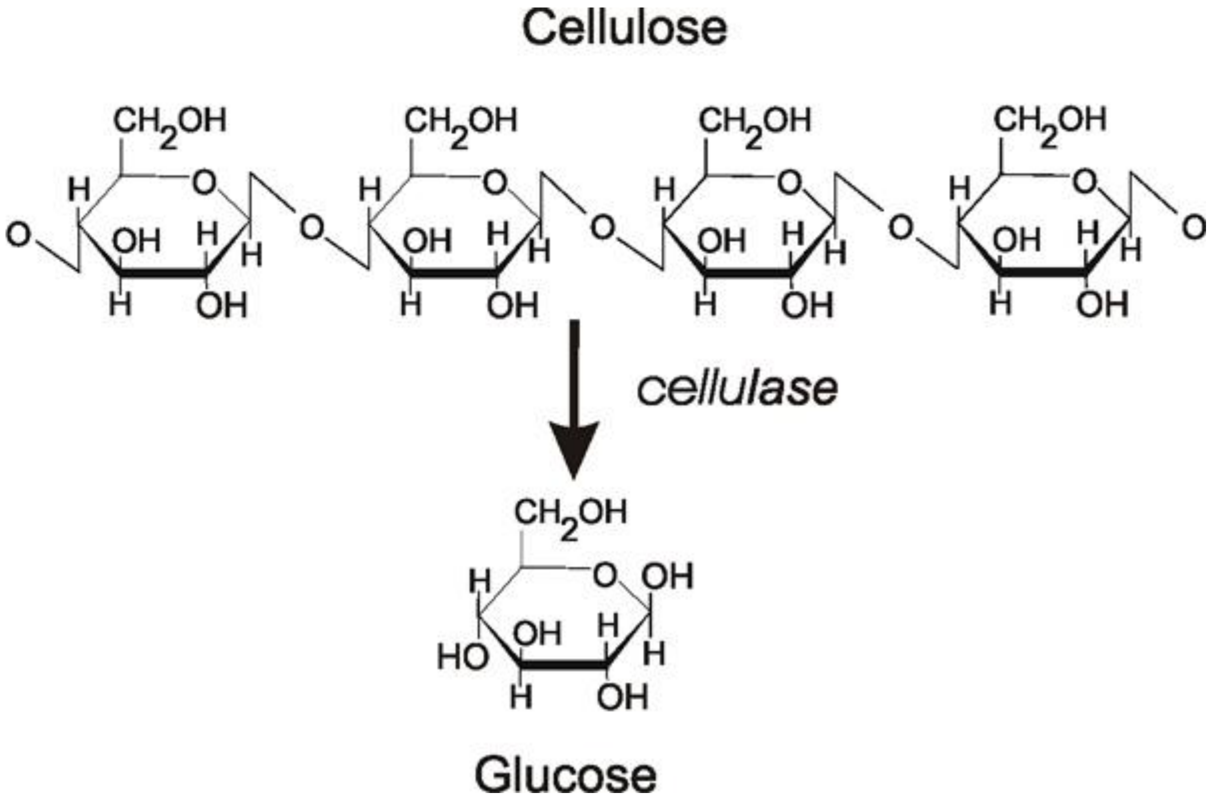


Ref:http://student.biology.arizona.edu/honors2003/group15/BackgroundTrans.htm

Hydrolysis


•Extracellular
enzymes
•Soluble substrates
•Insoluble
substrates
•Cell wall
•Intracellular
enzymes
•Cell membrane

Hydrolysis of cellulose



Ref:http://www.biotek.com/resources/articles/enzymatic-digestion-of-polysaccharides-2.html

Marmara Üniversitesi

Hydrolysis of Cellulose

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

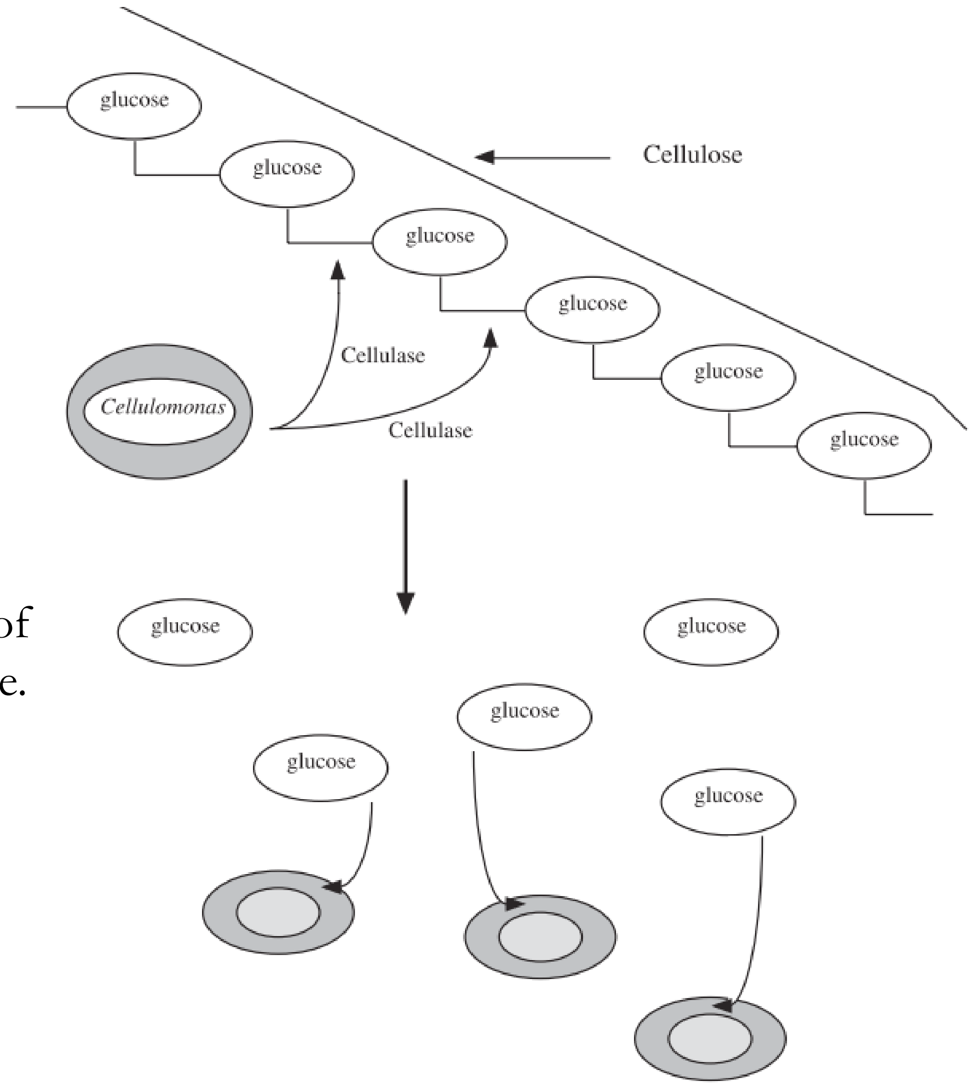
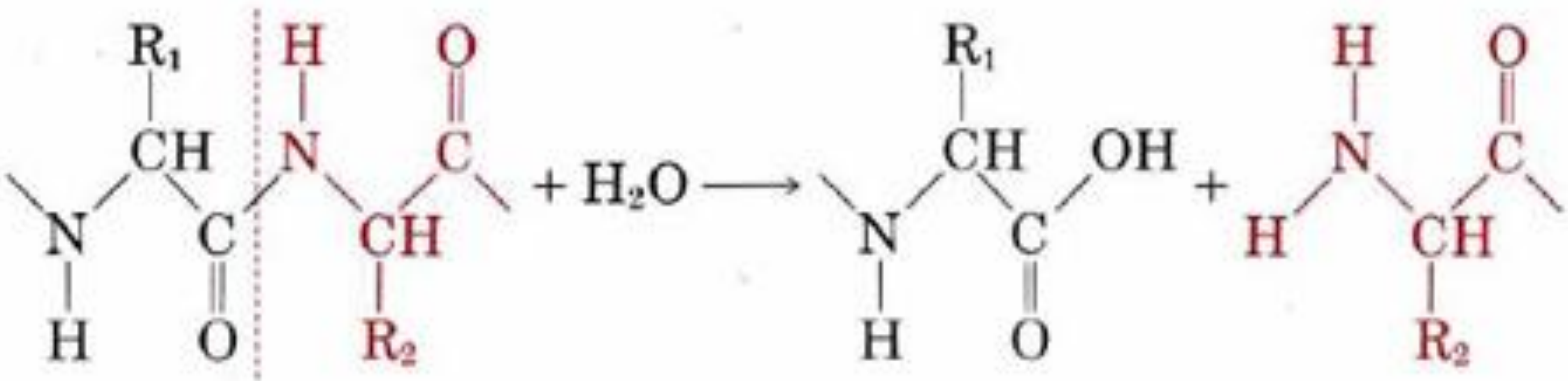


Figure 7.3 *Cellulose is an insoluble starch or particulate organic waste. Cellulose must be hydrolyzed before it can be degraded. Exoenzymes releases by specific hydrolytic bacteria such as* Cellulomonas *add water to the chemical bonds between the glucose units that make up cellulose. Once the chemical bonds are hydrolyzed, glucose goes into solution and is absorbed by numerous bacteria and degraded inside the bacterial cells.*

Hydrolysis of Proteins

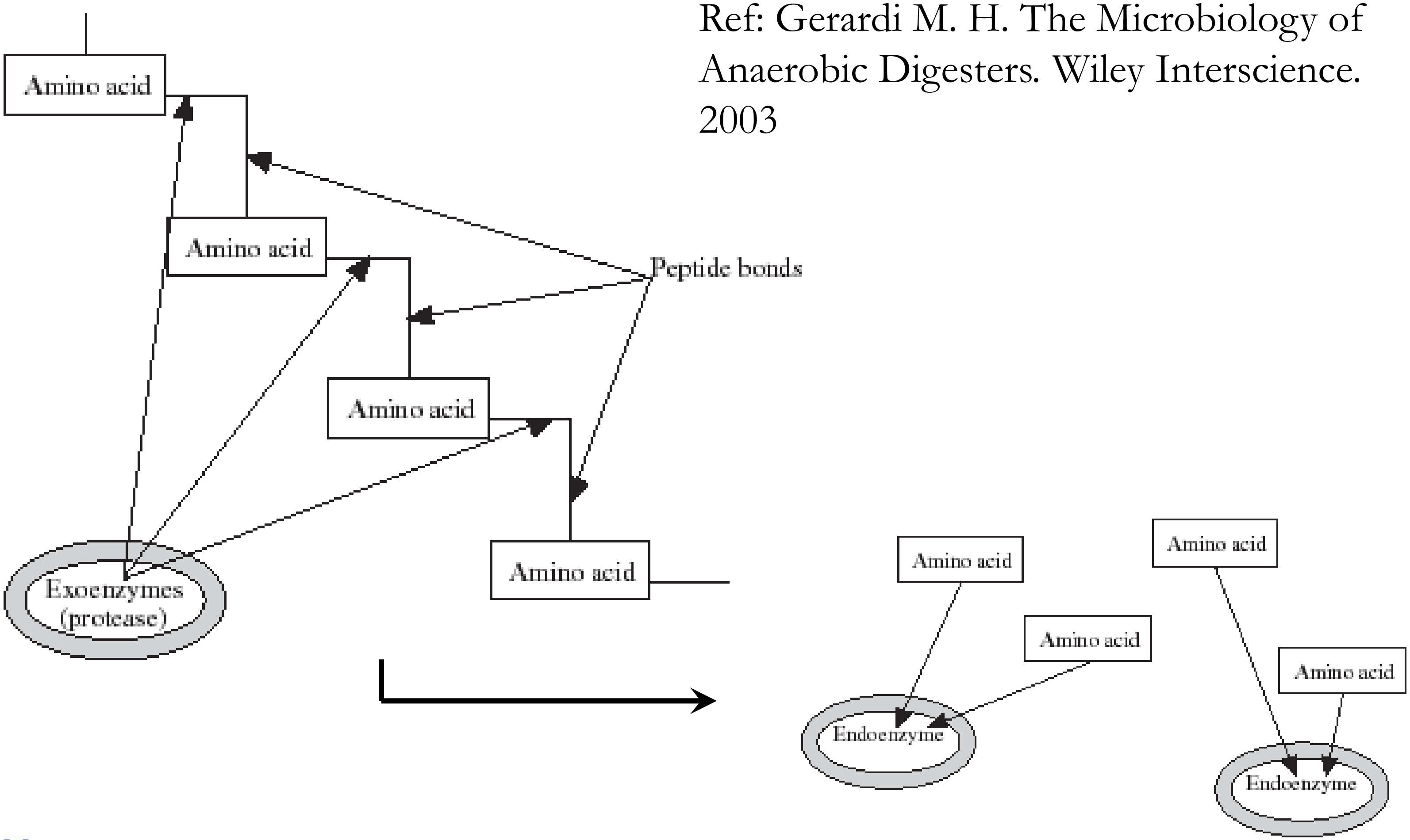
- Peptide



Ref:http://chempaths.chemeddl.org/services/chempaths/?q=book/General%20Chemistry%20Textbook/Chemical%20Kinetics/1789/enzymes

Hydrolysis of proteins

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003



Marmara Üniversitesi

Step 2 – Acid Forming Phase (Fermentation)

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*

Fermentative bacteria

Aeromonas	*Lactobacillus*
Bacteroides	*Pasteurella*
Bifidobacteria	*Propionobacterium*
Citrobacter	*Proteus*
Clostridium	*Providencia*
Enterobacter	*Salmonella*
Erwinia	*Serratia*
Escherichia	*Shigella*
Klebsiella	

Step 2 – Acid Forming Phase (Fermentation)

TABLE 7.1 Major Acids and Alcohols Produced Through Fermentation Processes in Anaerobic Digesters

Name	Formula
Acetate	CH_3COOH
Butanol	$CH_3(CH_2)_2CH_2OH$
Butyrate	$CH_3(CH_2)_2CH_2COOH$
Caproic acid	$CH_3(CH_2)_4COOH$
Formate	$HCOOH$
Ethanol	CH_3CH_2OH
Lactate	$CH_3CHOHCOOH$
Methanol	CH_3OH
Propanol	$CH_3CH_2CH_2OH$
Propionate	CH_3CH_2COOH
Succinate	$HOOCCH_2CH_2COOH$

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

Marmara Üniversitesi

Step 3 – Methane Forming Phase (Methanogenesis)

- Methane is mostly formed from acetate, H_2, and CO_2

Substrate	Chemical Formula
Acetate	CH_3COOH
Carbon dioxide	CO_2
Carbon monoxide	CO
Formate	$HCOOH$
Hydrogen	H_2
Methanol	CH_3OH
Methylamine	CH_3NH_2

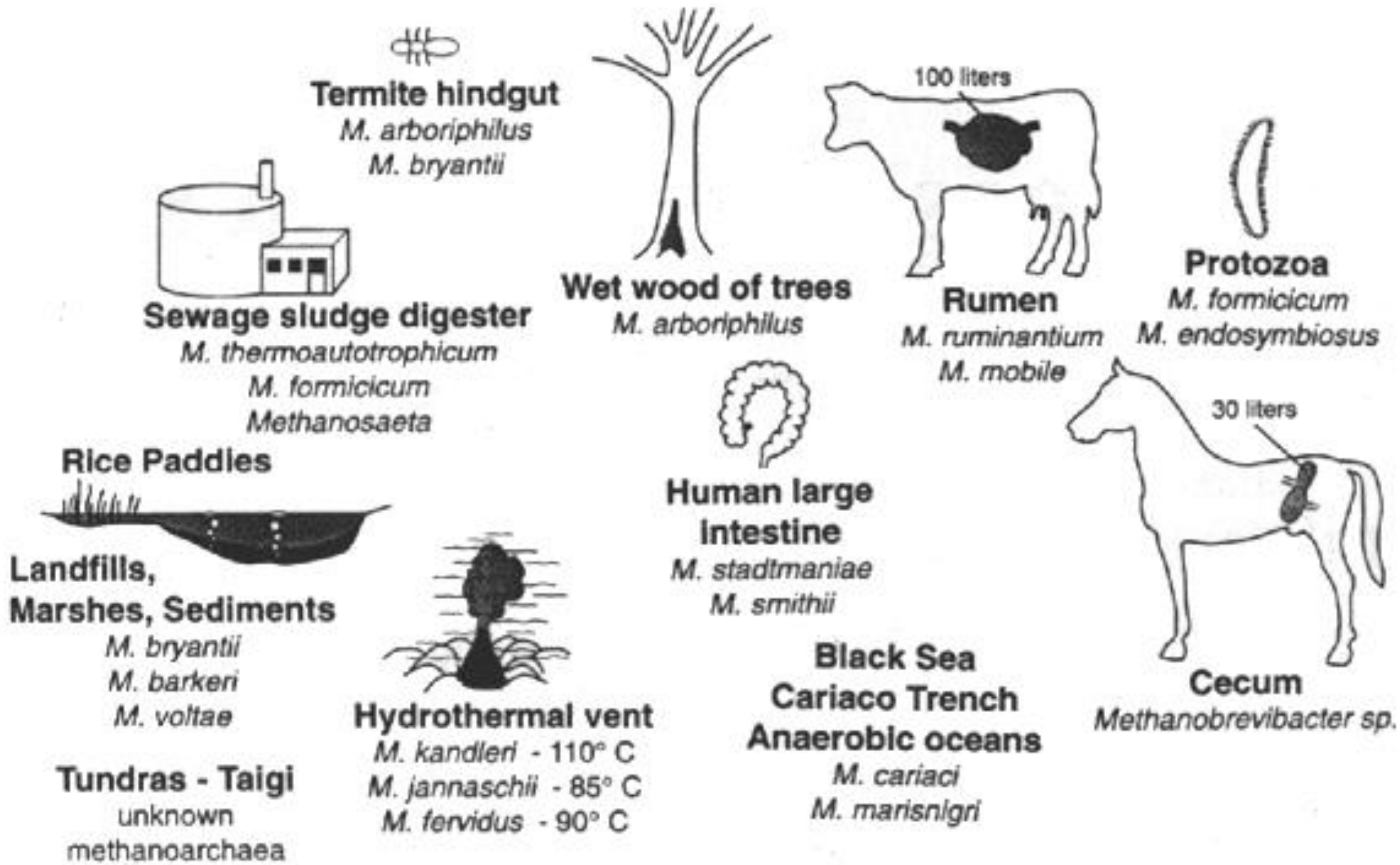
$$\text{Acetate} \xrightarrow{\text{acetoclastic methanogens}} CH_4 + CO_2$$

$$H_2 + CO_2 \xrightarrow{\text{hydrogenotrophic methanogens}} CH_4 + 2H_2O$$

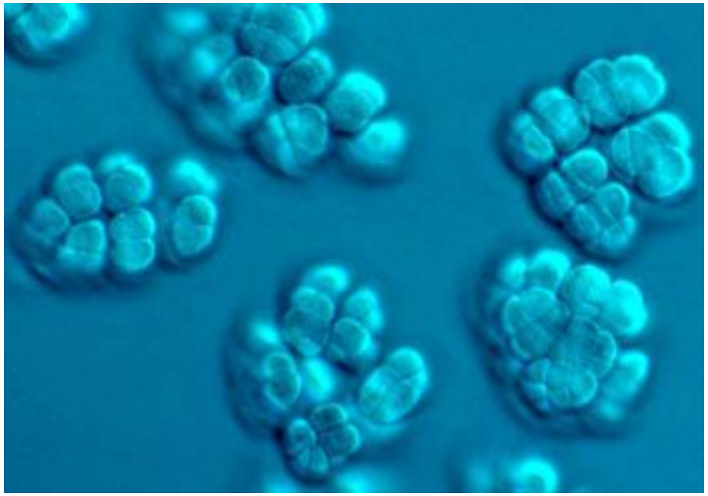
$$\text{Methanol} \xrightarrow{\text{methyltrophic methanogens}} 2CH_4 + 2H_2O$$

Ref: Gerardi M. H. The Microbiology of Anaerobic Digesters. Wiley Interscience. 2003

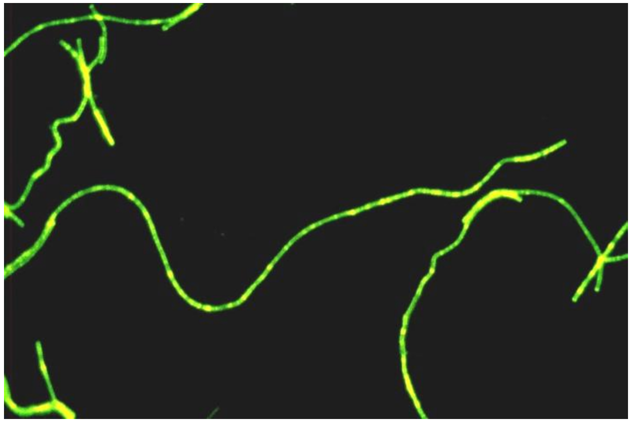
Habitats of methanogens

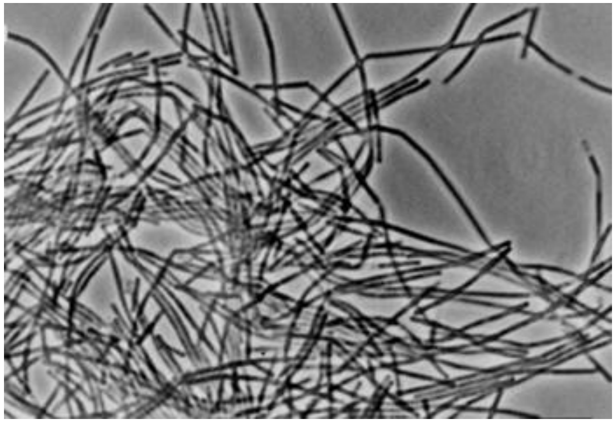


Acetoclastic Methanogens



- *Methanosarcina acetivorans*





- *Methanosaeta concilii*
- *Methanosaeta sp.*

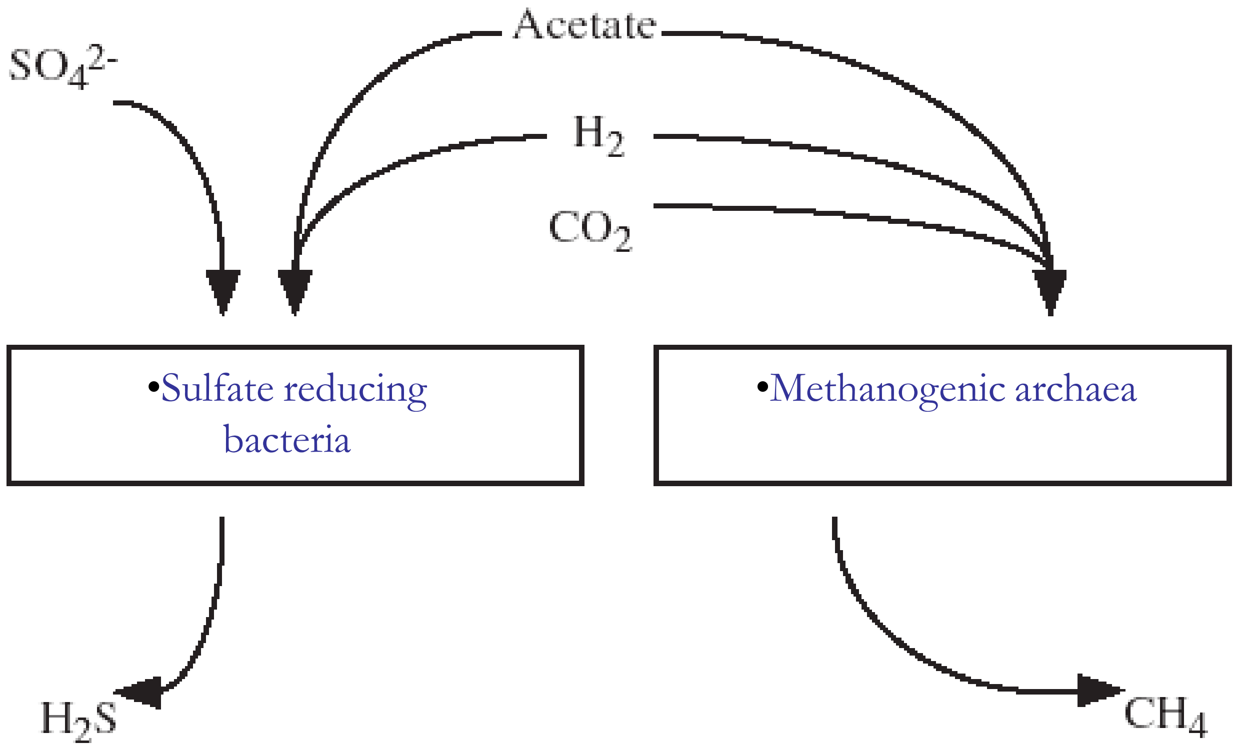
Methanogens

Methanosaeta
Substrate:
Acetate

Mixed culture
Substrate: Sucrose

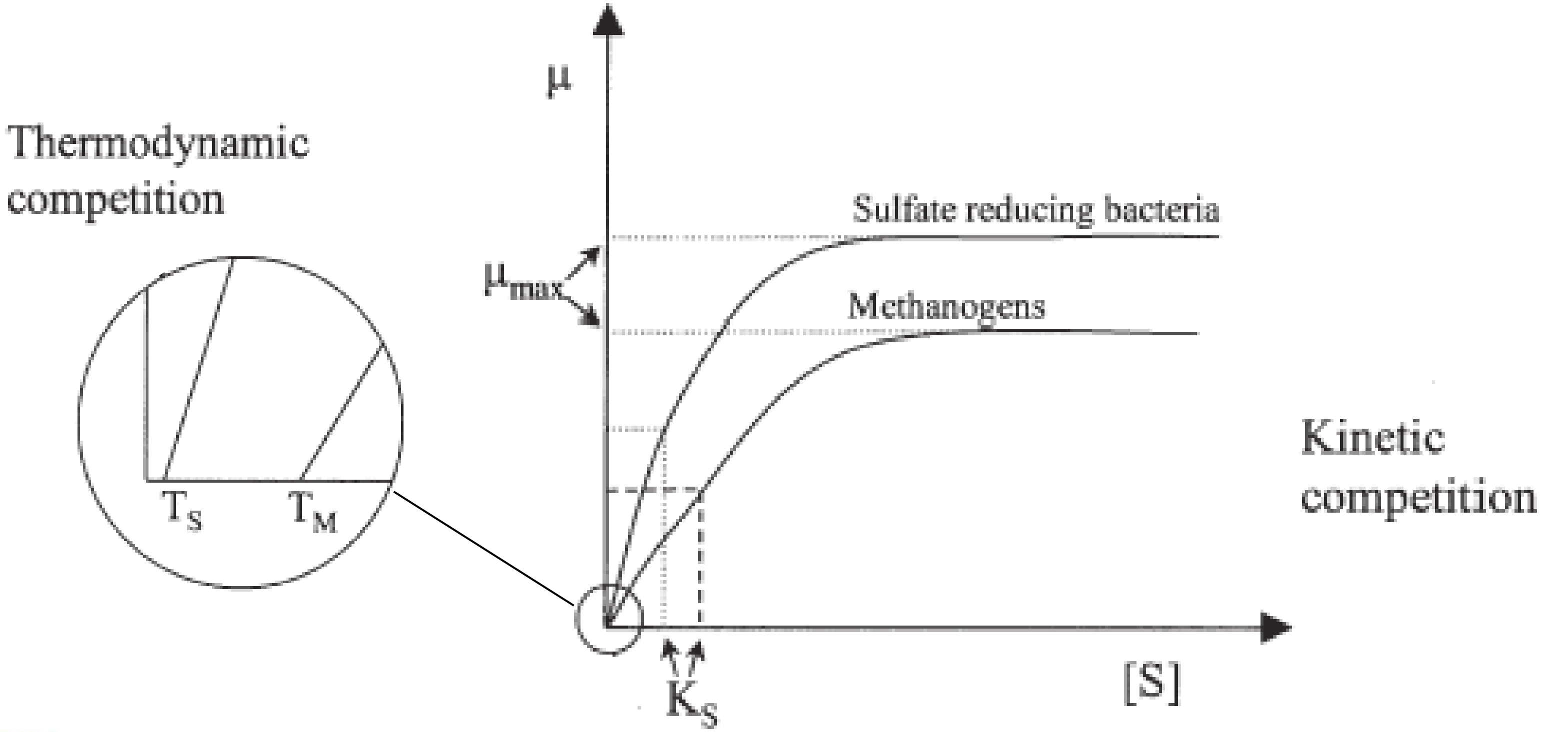
Marmara Üniversitesi

Competition: SRB vs Methanogens



Competition: SRB vs Methanogens

- Model of kinetic and thermodynamic competition among sulfate reducing bacteria and methanogenic archaea

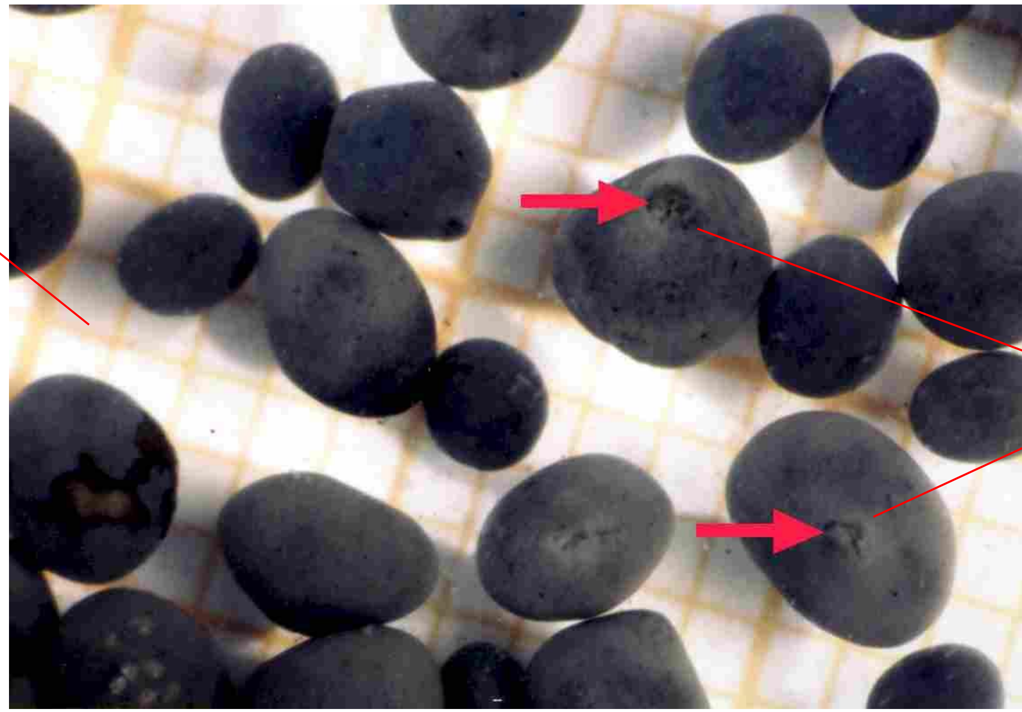


Minimum doubling times of major microbial groups in AD (Mosey, 1989)

Sugar-fermenting, acid forming bacteria	30	Min
Methanogens growing on hydrogen	6	Hours
Acetogenic bacteria fermenting butyrate	1.4	Days
Acetogenic bacteria fermenting propionate	2.5	Days
Methanogens growing on acetate	2.6	Days

Sludge Granulation

Millimeter paper indicating the size of the granules.

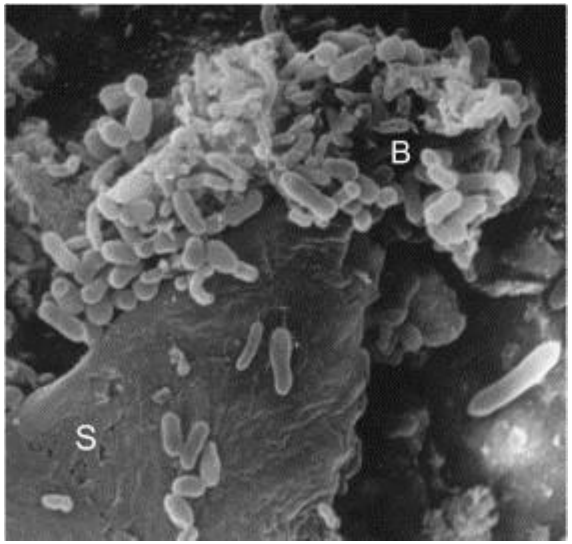


Gas vents in the granules, where biogas is released

Anaerobic sludge granules from a UASB reactor treating effluent from a recycle paper mill (Roermond, The Netherlands).

Marmara Üniversitesi

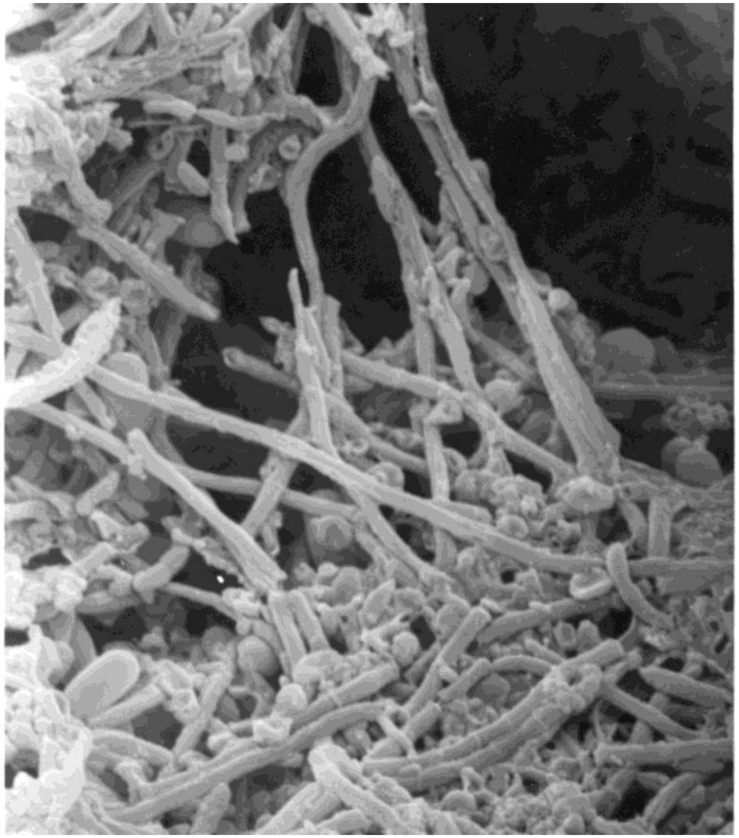
Arhael Biofilm



Ref: 1. Sossa, K.; Alarcón, M.; Aspé, E.; Urrutia, H. (2004) Effect of ammonia on the methanogenic activity of methylaminotrophic methane producing Archaea enriched biofilm. *Anaerobe, 10 (1): 13-18*.

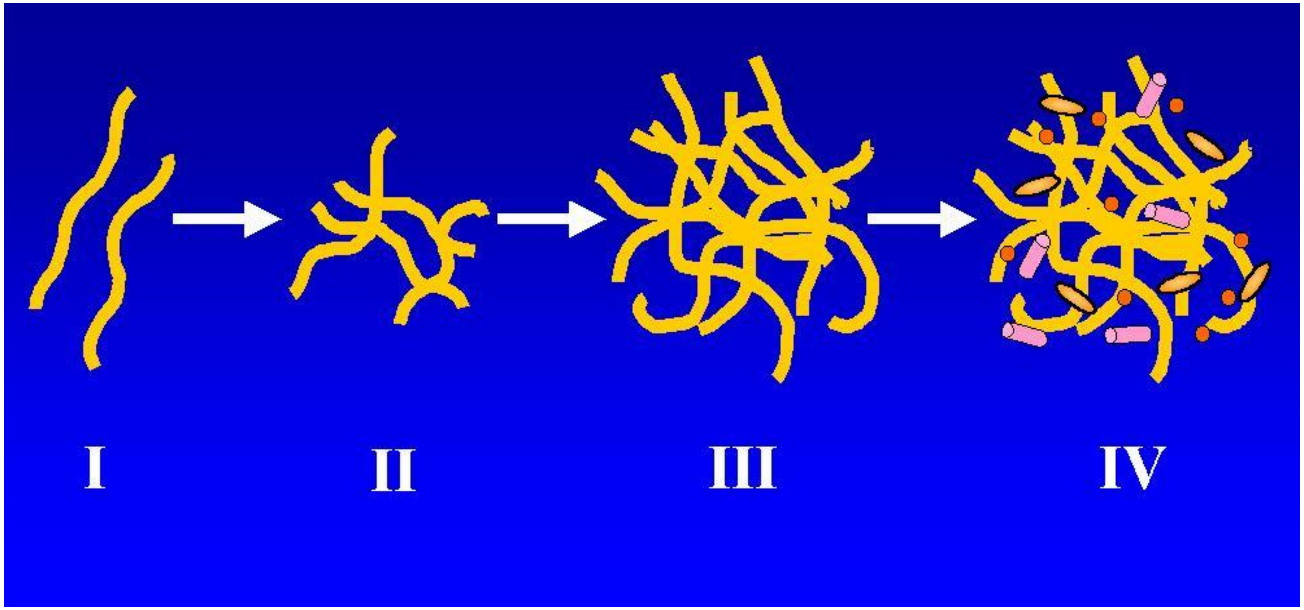
Marmara Üniversitesi

Archael Biofilm



Ref:
http://commtechlab.msu.edu/sites/dlcme/zoo/microbes/media/biofilm.jpg

Spaghetti theory of granulation



I) Disperse methanogens (filamentous *Methanosaeta*)
II) Floc formation via entanglement
III) Pellet formation (spaghetti balls) and
IV) Mature granules, with attachment of other anaerobic microorganisms onto the pellet.

Marmara Üniversitesi