Aalto University
School of Engineering

Biological treatment processes of water and waste
Lecture 2

WAT - E2180

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Lecture outline

Introduction to biodegradability

Measuring organic matter

Group discussion: Bioplastics and biodegradable plastics??

COD fractions

Toxicity

Introduction to biological processes

Classification of bacteria

Bacteria game

Stoichiometry

Energetics

Kinetics

Sumo demo

Biodegradability

- IUPAC definition: Degradation caused by enzymatic process resulting from the action of cells.
- = chemical dissolution of materials by bacteria, fungi or other biological means
- Generally organic material that serves as a nutrient for microorganisms = almost all organic compounds are subject to degradation.. The question is HOW FAST!!
- Biosurfactants = extracellular surfactants secreted by microorganisms, enhances the biodegradation process
- Small molecules break down faster
- Xenobiotics usually break down slowly

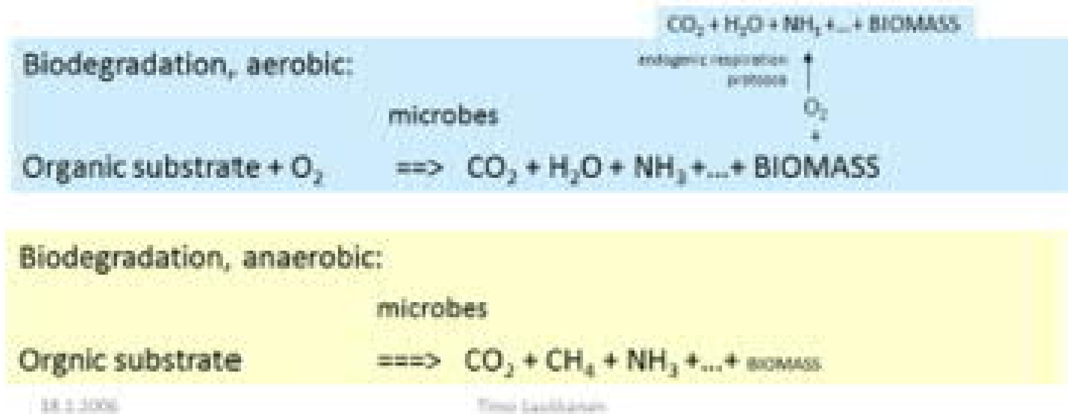
Time needed for biodegradation of different compounds

Approximated time for compounds to bio?degrade in a marine environment

Product	Time to Biodegrade	Product	Time to Biodegrade
Paper towel	2–4 weeks	Plywood	1-3 years
Newspaper	6 weeks	Painted wooden sticks	13 years
Apple core	2 months	Plastic bags	10–20 years
Cardboard box	2 months	Tin cans	50 years
Wax coated milk carton	3 months	Disposable diapers	50-100 years
Cotton gloves	1–5 months	Plastic bottle	100 years
Wool gloves	1 year	Aluminium cans	200 years
		Glass bottles	Undetermined

Aerobic and anaerobic biodegradation

- **Biodegradation can take place in aerobic or anaerobic conditions**
- **Aerobic = presence of O_2**
- **Anaerobic = absence of oxygen**
- **Biodegradation occurs as a result of microbial growth**



Hydrolysis

- Hydrolysis is the breakdown of a chemical bond by addition of water.
- Hydrolysis takes place when organic matter is in contact with water.
- Many microorganisms produce enzymes that catalyse the hydrolysis, e.g. proteases for proteins)
- These enzymes are selective.

Generic mechanism for a hydrolysis reaction.

Example of a biodegradation process

4

The decomposition of a organic monochloro compound

Complete decomposition:

$$H_yC_xO_zCl + (x\text{-}z/2+(y\text{-}1)/4)\ O_2 \rightarrow x\ CO_2 + (y\text{-}1)/2\ H_2O + H^+ + Cl^-$$

Dechlorination:

$$H_yC_xO_zCl + m\ O_2 \rightarrow (x\text{-}n)\ CO_2 + n\ Org.C + Cl^- + \ldots$$

No dechlorination:

$$H_yC_xO_zCl + m'\ O_2 \rightarrow (x\text{-}n)\ CO_2 + n\ Org.C + Org.Cl + \ldots$$

What to measure?

- Oxygen uptake, BOD
- Decrease of organic carbon
- Decrease of organic chlorine
- Increase of chloride

Group discussion: Bioplastics and biodegradable plastics?

Seach for information about bioplastics and biodegradable plastics

Discuss in groups what these terms actually mean

Discuss about pros and cons about both

(15 – 20 min)

How do we measure organic matter

Organic matter contains all kinds of different organic substances, also living cells but ≠ biomass
Organic matter = food, biomass = the ones who eat

TOC = total organic carbon, mgC/l

(most common method catalytic oxidation)

DOC = dissolved organic matter

(NOM = natural organic matter)

BOD = biological oxygen demand (5 or 7 days)

mg O_2/l

(incubation method)

COD = chemical oxygen demand

ThOD = theoretical oxygen demand (mg O_2/g)

SBCOD = slowly biodegradable

RBCOD = readily biodegradable

Etc.

Theoretical oxygen demand

- Theoretical COD is the calculated amount of oxygen needed to oxidize a compound to its final oxidation products.
- Note: some differences between standard methods on how nitrogen is dealt with.

Steps:

1) Calculate the carbonaceous oxygen demand in moles(based on the oxidation state of carbon)
2) Calculate nitrogenous oxygen demand in moles
3) Calculate ThOD (gO_2/g) using molar masses

Example: Calculation of ThOD

$x*2*(-2-0) = x*(-4)$

O.S. $\quad z \quad 0 \quad\quad +4 \;\; -2$

$$\text{Org-C} + x \cdot O_2 \;\text{-->}\; C\,O_2 + ...$$

$z-4$

$$4-z + x*(-4) = 0 \quad => \quad x = (4-z)/4$$

Example: Ethanol

O.S. $\quad +1 \;\; z \;\; -2 \quad\quad 0 \quad\quad +4 \;\; -2$

$$H_5C_2OH + 2x \cdot O_2 \;\text{-->}\; 2*C\,O_2 + ...$$

$$2z + (+1*6) + (-2) = 0 \quad => \quad z = -2$$

$$=> x = (4-(-2))/4 = 1½$$

What is the ThOD [g-O_2/g-Ethanol]?

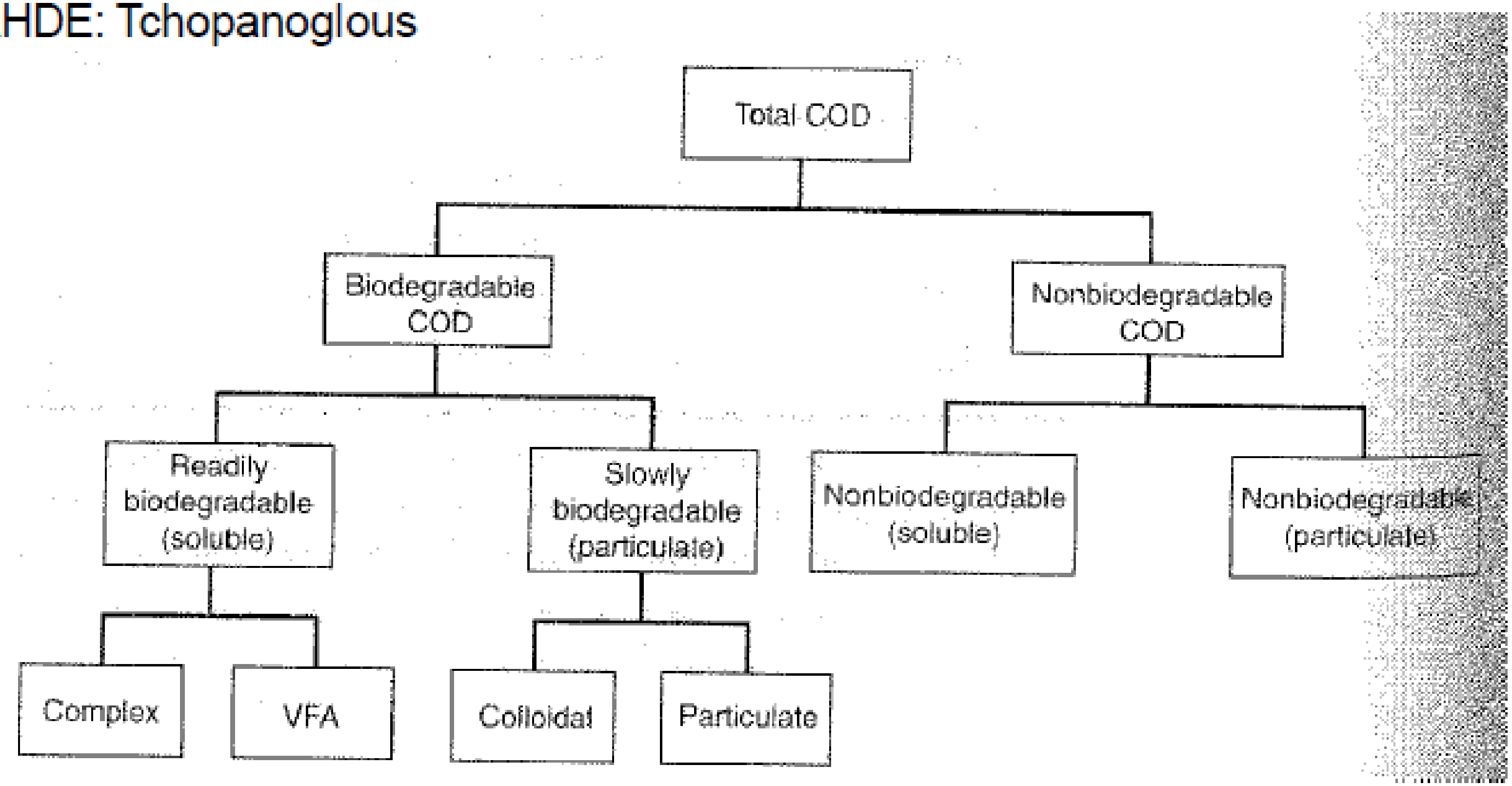
ThOD = 3*2*16 g-O_2/mol / (6*1+2*12+16) g-Ethanol/mol = 2.8 g-O_2/g-Ethanol

Examples of different oxidation states of carbon

Substance		Oxidn. state of Carbon	x		ThOD $g\text{-}O_2/g$		ThOD/ TOC
Methane CH_4	H_4C	-4	2	(4*16)/(4+12)=	2	4*16/12=	5,3
Methanol H_3COH	H_4CO	-2	1½	(3*16)/(4+12+16)=	1½	3*16/12=	4
Formalin H_2CO	H_2CO	0	1	(2*16)/(2+12+16)=	1	2*16/12=	2,7
Formic acid HCOOH	H_2CO_2	+2	½	16/(2+12+2*16)=	½	16/12=	1,3

Characterization of the organic matter

LÄHDE: Tchopanoglous



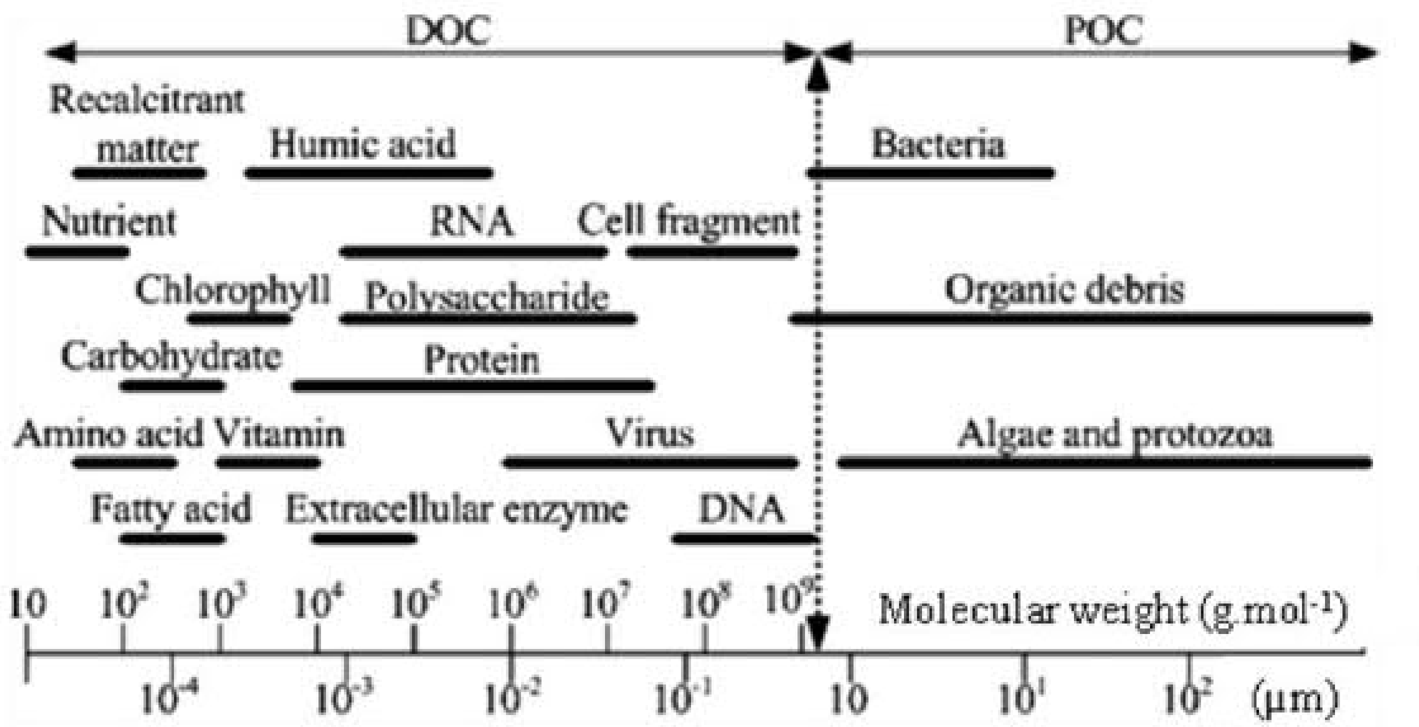


Figure 1: Organic Components in Traditional Wastewater Treatment Plant Effluent. Adapted from Shon et al. (2006).

Toxicity and inhibition

- **Biological processes deal with living organisms → affected by inhibitory or toxic agents**
- **Inhibition = impairment of the enzymatic system or damage to cell structure**
- **Toxic effect = inhibition is caused to a vital activity**
- **Inhibition can be caused by pH, temperature, redox limitations**
- **A medium is not either toxic or non-toxic → it's a continuum**
- **Acclimatisation can be used to decrease the inhibitory effects**
- **Ways of measuring toxicity:**
  - Respirometry
  - Bioluminescence (Vibrio fischeri)
  - Many other bioassays

Introduction to biological processes

Metabolism of living cells

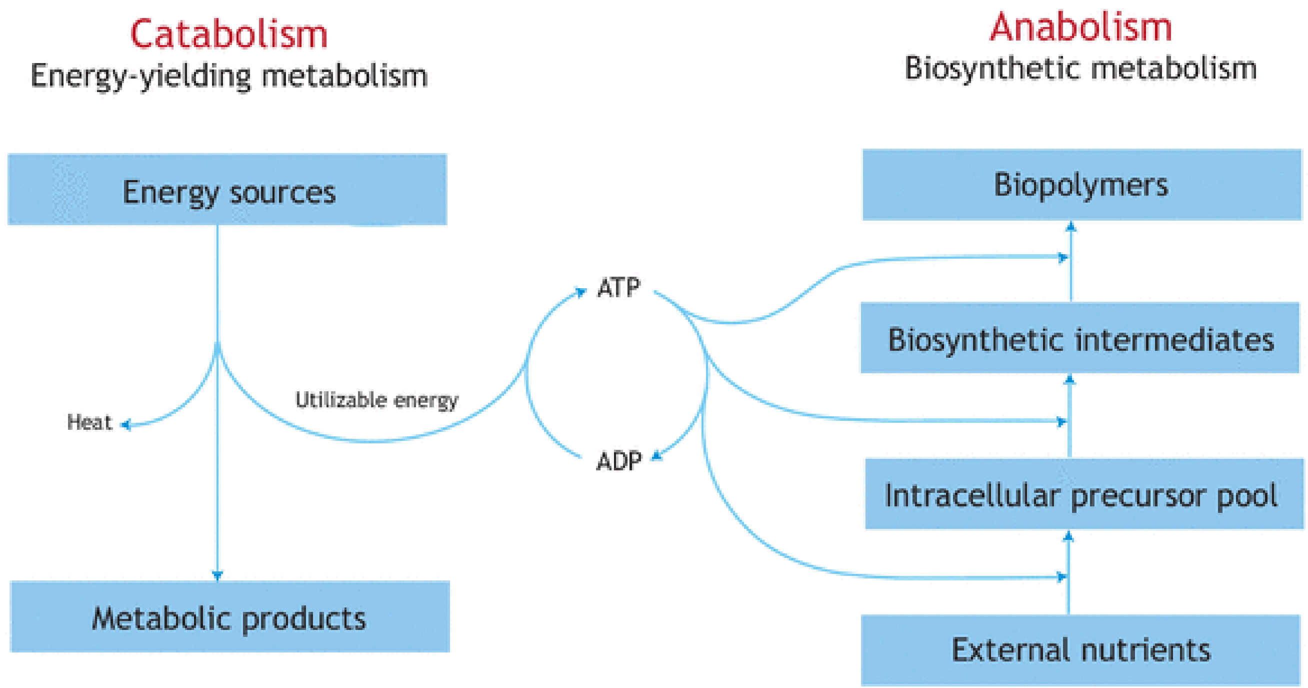
Catabolism = energy supply of the cell (redox reaction)

Anabolism = synthesis of cellular components from carbon sources and other nutrients

Main requirements

→ Electron donor and acceptor

→ Carbon source



How do bacteria obtain energy?

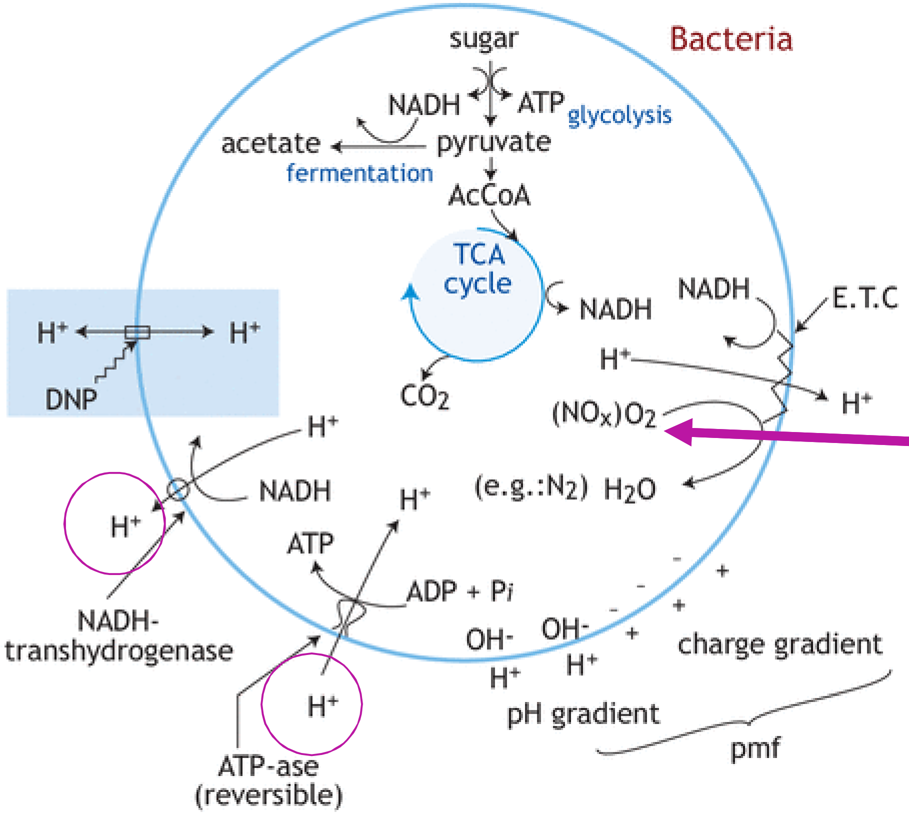


Figure 2.13 Overview of bacterial bioenergetics (adapted from Comeau *et al.*, 1986)

Main pathway for energy production in cells:

Glycolysis + Krebs cycle or TCA cycle

Requires an electron acceptor!

You can watch a video of the TCA cycle

https://www.youtube.com/watch?v=lV9X2K8uEYE

Classification of bacteria

Carbon source	Energy source	Relationship to oxygen	Temperature
- Autotrophs - Heterotrophs	- Phototrophs (Light) - Lithotrophs (inorganic) - Organotrophs (organic) chemical compounds	- Aerobic - Anaerobic - Facultative	- Psychrophilic - Mesophilic - Thermophilic

Auto= self, hetero = others, photo = light, chemo = chemical, troph = nourishment, litho = inorganic, organo = organic

Table 2.5 Engineering definition of some environmental conditions

Condition		Electron acceptor	
		Present	Absent
Aerobic	OX	O_2	
Anoxic	AX	NO_x	O_2
Anaerobic	AN		O_2 and NO_x

NO_x refers to nitrate (NO_3^-) plus nitrite (NO_2^-)

Examples of reactions

- *Aerobic heterotrophs:* organic matter oxidation

$$C_6H_{12}O_6 + O_2 + NH_3 + other\ nutrients \rightarrow C_5H_7O_2N + CO_2 + H_2O \quad (2.1)$$

- *Denitrifiers:* nitrate removal

$$C_6H_{12}O_6 + O_2 + HNO_3 + NH_3 + other\ nutrients \rightarrow C_5H_7O_2N + CO_2 + H_2O + N_2 \quad (2.2)$$

- *Aerobic autotrophic bacteria (ammonia oxidizers):* removal of ammonia

$$CO_2 + NH_3 + O_2 + other\ nutrients \rightarrow C_5H_7O_2N + HNO_3 + H_2O \quad (2.4)$$

- *Hydrogenotrophic methanogens:* biogas production

$$H_2 + CO_2 + NH_3 + other\ nutrients \rightarrow C_5H_7O_2N + CH_4 \quad (2.5)$$

Microorganismsin water and wastewater treatment

Table 2.3 Trophic classification of microorganisms (adapted from Rittmann and McCarty, 2001; Metcalf & Eddy, 2003)

Energy source					Carbon source[1]
Electron donor			Electron acceptor	Typical products[2]	
Trophic group	Microbial group	Type of e^- donor			
Chemotroph					
Organotroph	Aerobic heterotrophs	Organic	O_2	CO_2, H_2O	Organic
	Denitrifiers	Organic	NO_3^-, NO_2^-	N_2, CO_2, H_2O	Organic
	Fermenting organisms	Organic	Organic	Organic:VFAs[3]	Organic
	Iron reducers	Organic	Fe (III)	Fe (II)	Organic
	Sulfate reducers	Acetate	SO_4^{2-}	H_2S	Acetate
	Methanogens (acetoclastic)	Acetate	acetate	CH_4	Acetate
Lithotroph	Nitrifiers: AOB[4]	NH_4^+	O_2	NO_2^-	CO_2
	Nitrifiers: NOB[5]	NO_2^-	O_2	NO_3^-	CO_2
	Anammox[6] bacteria	NH_4^+	NO_2^-	N_2	CO_2
	Denitrifiers	H_2	NO_3^-, NO_2^-	N_2, H_2O	CO_2
	Denitrifiers	S	NO_3^-, NO_2^-	N_2, SO_4^{2-} H_2O	CO_2
	Iron oxidizers	Fe (II)	O_2	Fe (III)	CO_2
	Sulphate reducers	H_2	SO_4^{2-}	H_2S, H_2O	CO_2
	Sulphate oxidizers	H_2S, S^0,$S_2O_3^{2-}$	O_2	SO_4^{2-}	CO_2
	Aerobic hydrogenotrophs	H_2	O_2	H_2O	CO_2
	Methanogens (hydrogenotrophic)	H_2	CO_2	CH_4	CO_2
Phototroph					
	Algae, plants	H_2O	CO_2	O_2	CO_2
	Photosynthetic bacteria	H_2S	CO_2	S (0)	CO_2

[1] Carbon source: organic for heterotrophs and inorganic (CO_2) for autotrophs; mixotrophs can use both. [2] Typical products: CO_2 and H_2O are products of catalysis (energy generation) by many micro-organisms. [3] VFAs: volatile fatty acids (typically acetate, propionate, butyrate).
[4] AOB: ammonia oxidizing bacteria. [5] NOB: nitrite oxidizing bacteria. [6] Anammox: anaerobic ammonia oxidizing bacteria.

Ready for BACTERIA GAME?

- Each student takes a card
- Four of you will be bacteria
- Form groups with beneficial conditions for each bacteria

Stoichiometry

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$180\ g \qquad 192\ g$$

↓

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+$$

↓

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O \rightarrow \frac{1}{4}CO_2 + e^- + H^+$$

+

$$0.25O_2 + H^+ + e^- \rightarrow 0.5H_2O$$

Important:

- **Electron equivalents need to be kept**
- **Charge balance by adding protons**
- **Water for H balance**

Cell growth

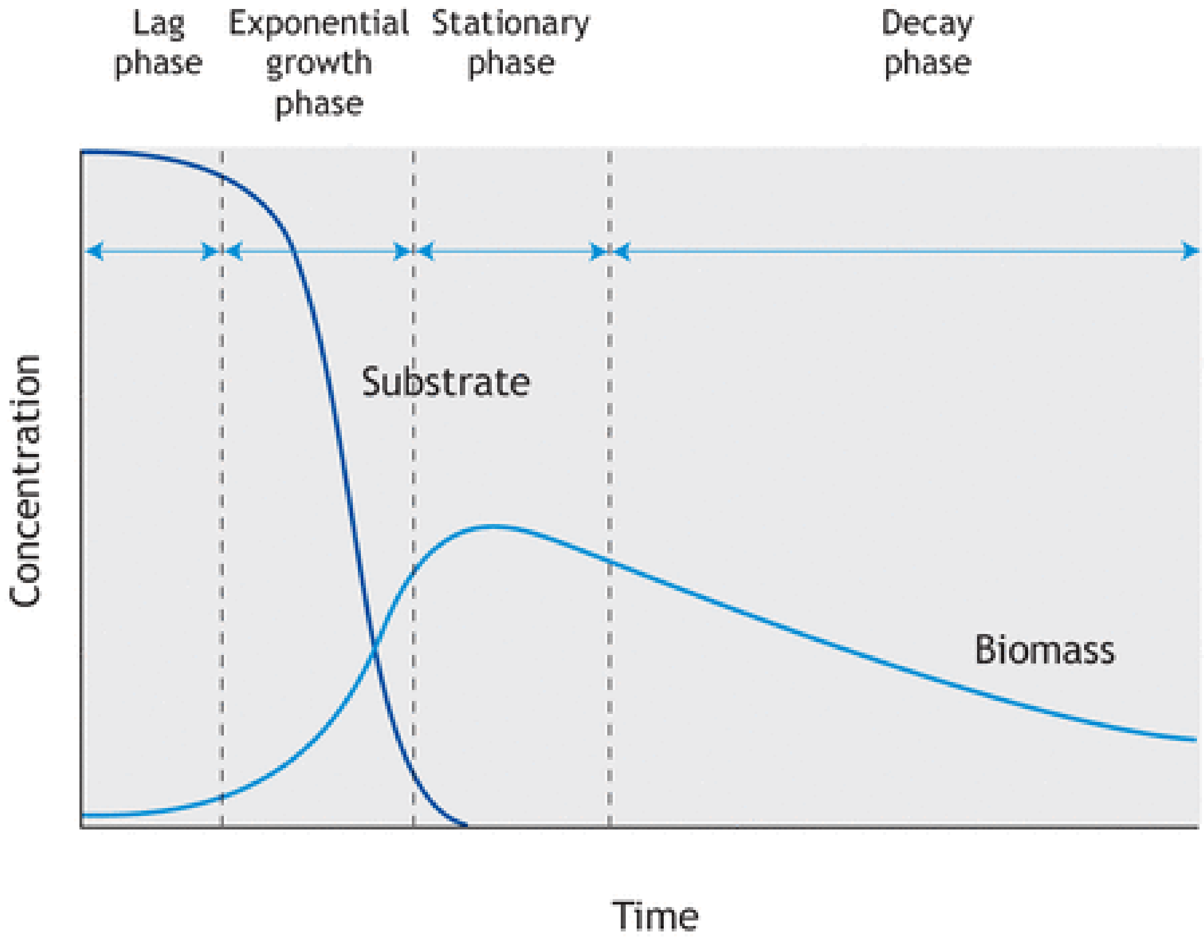


Figure 2.16 Biomass growth in batch mode (adapted from Metcalf & Eddy, 2003)

Microbial growth

Growth can be described with the equation:

$$r_{V,XB} = \mu_{max} \; f(S) \; X_B$$

Where

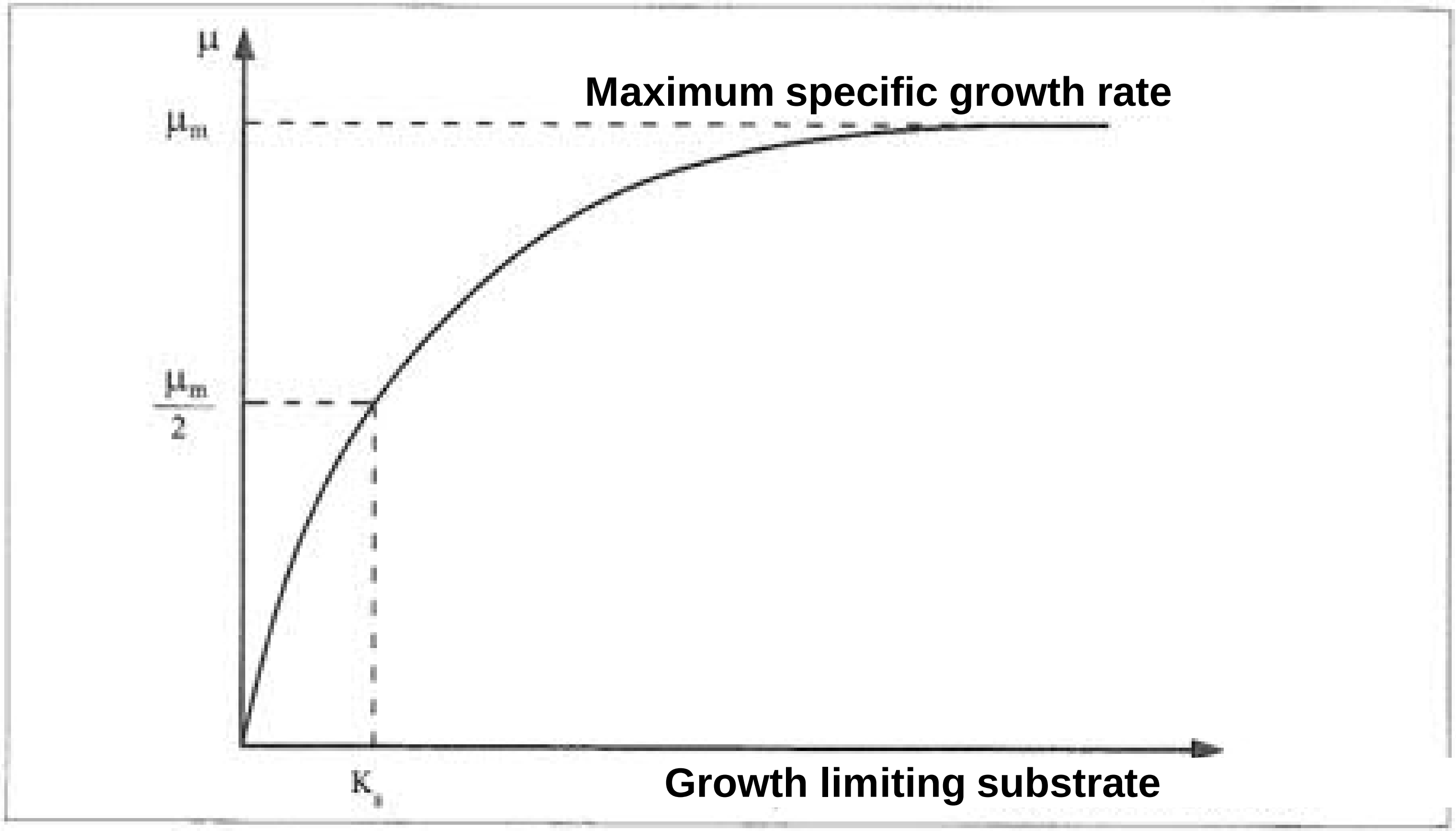
$r_{V,XB}$ = growth per unit of volume and time (e.g. kgCOD/m^3*d)

μ_{max} = max specific growth rate (1/h or 1/d)

F(S) = growth kinetic function (depending on substrate), typically Monod

X_B = biomass concentration (kgCOD/m^3 or kgVSS/m^3)

Monod’s kinetics



Substrate consumption

$$r_{V,S} = r_{V,B} / Y_{max}$$

Y_{max} = maximum yield (kgCOD(B)/kgCOD(S) or kgVSS(B)/kgCOD(S)

Yield shows how much of the consumed substrate is transformed into new biomass in the reaction.

Note also Y_{obs} which is smaller than Y_{max}

Monod kinetics

$$r_{V,XB} = \mu_{max} \cdot \frac{S}{S + K_S} \cdot X_B$$

$$r_{V,S} = \frac{\mu_{max}}{Y_{max}} \cdot \frac{S}{S + K_S} \cdot X_B$$

$$\mu_{obs} = \mu_{max} S / (S + K_s) \quad [1/d]$$

Monod kinetics are typically used for microbial growth

For substrate consumption (g/m^3d)

For biomass growth

(g/m^3d)

Observed specific growth rate

Taking into account the growth conditions

Oxygen:

$$\mu_{obs} = \mu_{\max} \cdot \frac{S_{O2,2}}{S_{O2,2} + K_{S,O2}}$$

$$\mu_{obs} = \mu_{\max} \cdot \frac{S_2}{S_2 + K_S} \cdot \frac{S_{O2,2}}{S_{O2,2} + K_{S,O2}}$$

Temperature:

$$\mu_{\max(T)} = \mu_{\max(20°C)} \cdot e^{K(T-20)}$$

Typical values for stoichiometric and kinetic parameters

Table 2.9 Typical values of stoichiometric (f_S^o, Y) and kinetic (q_{max}, μ_{max}) parameters for various bacterial groups, (adapted from Rittmann and McCarty 2001)

Electron donor		Electron acceptor	f_S^0	Y	μ_{max}	K
Microbial group	e^- donor					
Chemotrophic organotrophs						
Aerobic heterotrophs	Sugar	O_2	0.70	0.49 gVSS/gbCOD	13.2	27.0 g bCOD/gVSS.d
Aerobic heterotrophs	No sugar	O_2	0.60	0.42 gVSS/gbCOD	8.4	17.0 g bCOD/gVSS.d
Denitrifiers	Organic	NO_3^-, NO_2^-	0.50	0.25 gVSS/gbCOD	4.0	16.0 g bCOD/gVSS.d
Fermenting organisms	Sugar	Organic	0.18	0.18 gVSS/gbCOD	1.2	10.0 g bCOD/gVSS.d
Sulphate reducers	Acetate	SO_4^{2-}	0.08	0.057 gVSS/gbCOD	0.5	8.7 g bCOD/gVSS.d
Methanogens (acetoclastic)	Acetate	Acetate	0.05	0.035 gVSS/gbCOD	0.3	8.4 g bCOD/gVSS.d
Chemotrophic lithotrophs						
Nitrifiers :AOB	NH_4^-	O_2	0.14	0.34 gVSS/gNH_4-N	0.9	2.7 g NH_4-N /gVSS.d
Nitrifiers :NOB	NO_2^-	O_2	0.10	0.08 gVSS/gNO_2-N	0.5	1.1 g NO_2-N/gVSS.d
Methanogens (hydrogenotrophic)	H_2	CO_2	0.08	0.45 gVSS/gH_2	0.3	1.1 g H_2/gVSS.d

bCOD: biodegradable COD
μ_{max} in gVSS /gVSS d
k = μ_{max}/Y= specific r_{max} (per unit biomass)

Occurence of biological processes in water and wastewater systems

Networks:

- **Drinking water pipes**
  - Consumption of NOM
  - Implications of e.g. dosing of desinfectants
- **Sewer system**
  - Biofilms allow longer retention time in the sewer for bacteria and for substrates

Drinking water:

  - Some processes
  - Removal of organic matter
  - Removal of e.g. pesticides
- **Wastewater treatment**
  - Most common and most feasible process for removal of soluble organic matter and nitrogen
  - Several different processes and configurations