Aalto University
School of Engineering

Biological treatment processes of water and waste
Lecture 3

WAT - E2180

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Lecture outline

Introduction to biodegradability

Measuring organic matter

Group discussion: Bioplastics and biodegradable plastics??

Understand the idea of biodegradability and its impact on biological processes

Wastewater characteristics / COD fractions

Toxicity

DEMO on COD fractionation

Understand some possible methods to assess biodegrability

Introduction to biological processes

Classification of bacteria

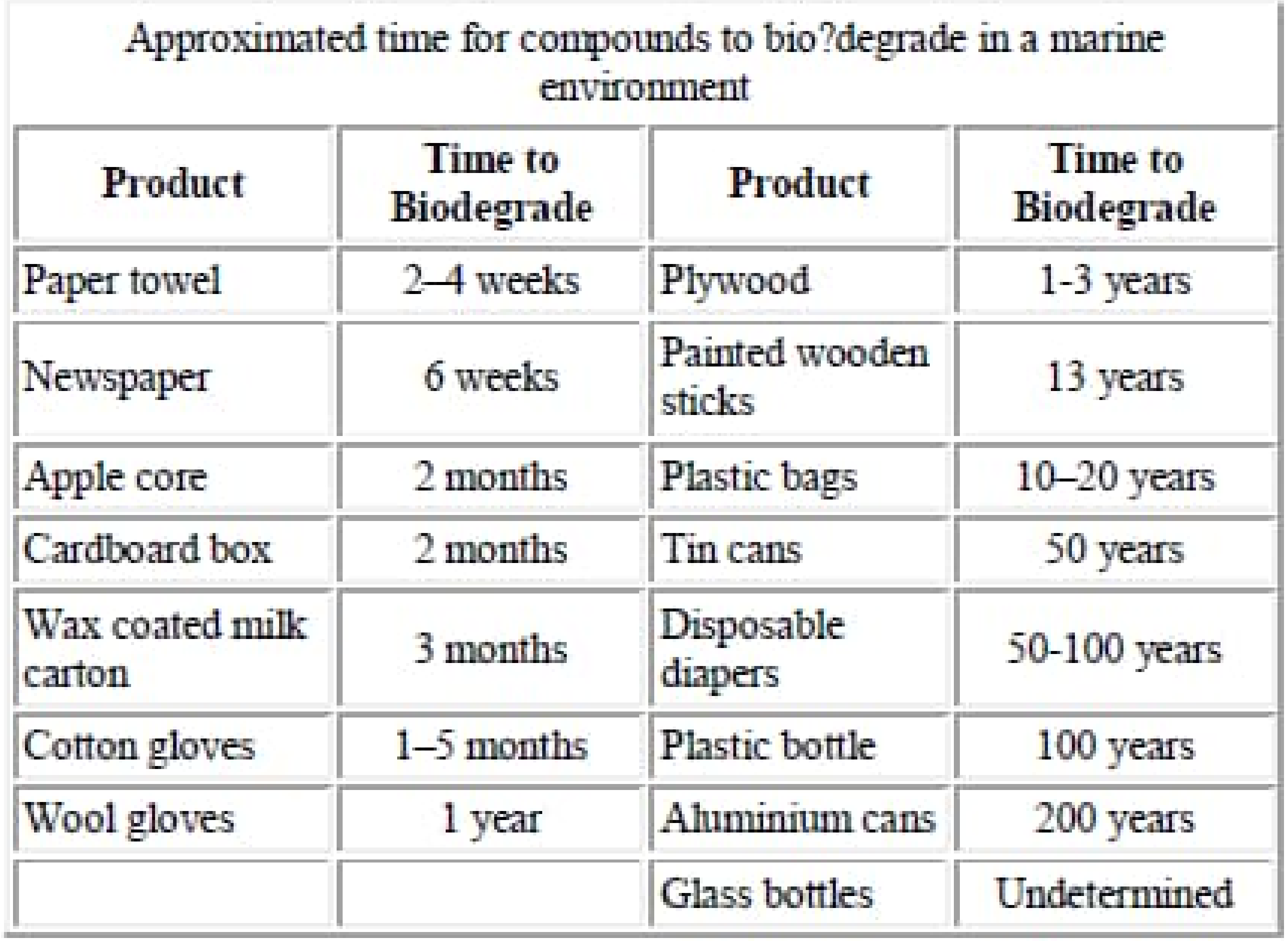
Bacteria game

Introduction to biological processes

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 all organic material that serves as a nutrient for microorganisms is biodegradable** = 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

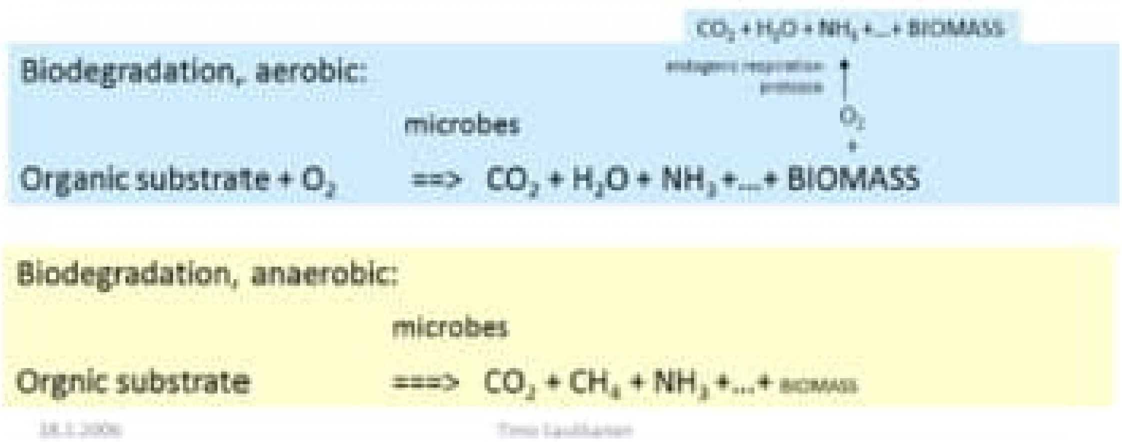


Approximated time for compounds to bio?degrade in a marine environment

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

Group discussion: Bioplastics and biodegradable plastics?

Seach for information about bioplastics and biodegradable plastics (5 min)

Discuss in groups what these terms actually mean (10 min)

Common discussion about your findings (5 min)

BREAK 10 min

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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, mgC/l

(NOM = natural organic matter)

BOD = biological oxygen demand (5 or 7 days), mg O_2/l

(incubation method)

COD = chemical oxygen demand, mg O_2/l

ThOD = theoretical oxygen demand, mgO_2/g

SBCOD/RBCOD = slowly/readily biodegradable

?

This Photo by Unknown

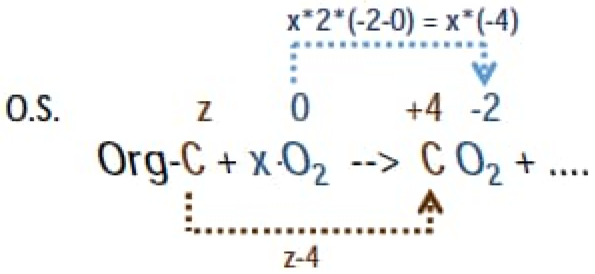
Theoretical oxygen demand

- **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. $\quad z \quad 0 \quad +4 \quad -2$

$$\overset{z}{\text{Org-C}} + x\cdot\overset{0}{O_2} \dashrightarrow \overset{+4}{C}\,\overset{-2}{O_2} + \ldots.$$

$z-4$

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

Example: Ethanol

O.S. $\quad \overset{+1\;z\;-2}{H_5C_2OH} + 2x\cdot\overset{0}{O_2} \dashrightarrow 2*\overset{+4}{C}\,\overset{-2}{O_2} + \ldots.$

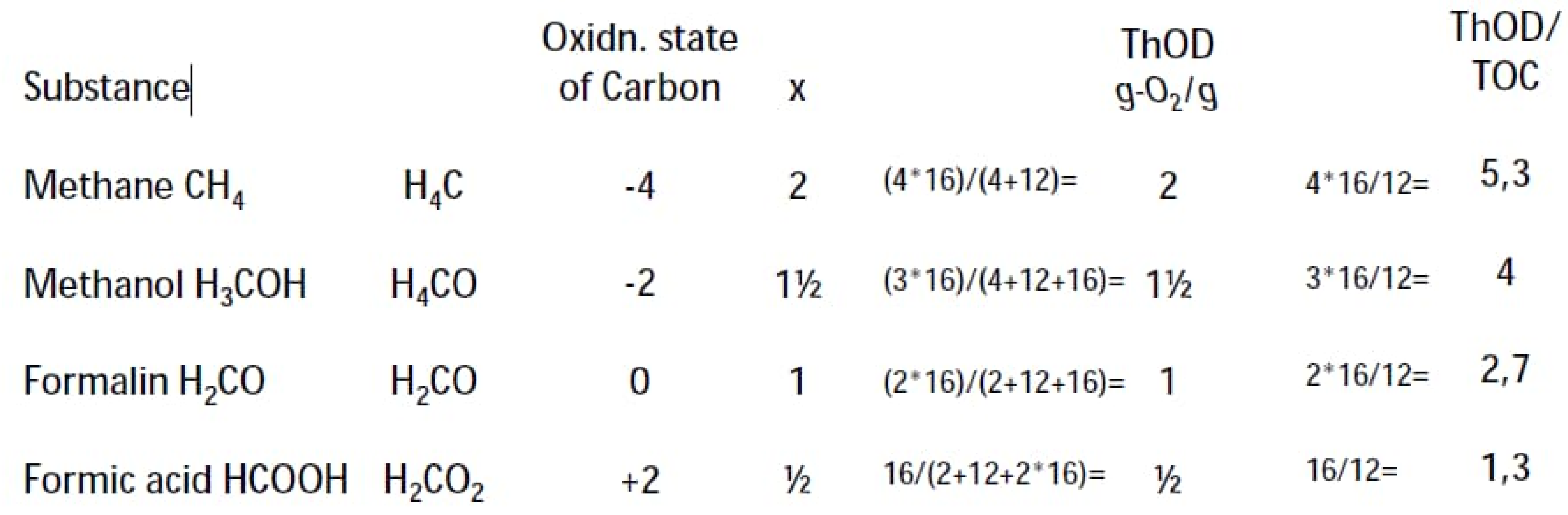
$$2z + (+1*6) + (-2) = 0 \quad \Rightarrow \quad z = -2$$

$$\Rightarrow x = (4-(-2))/4 = 1\tfrac{1}{2}$$

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

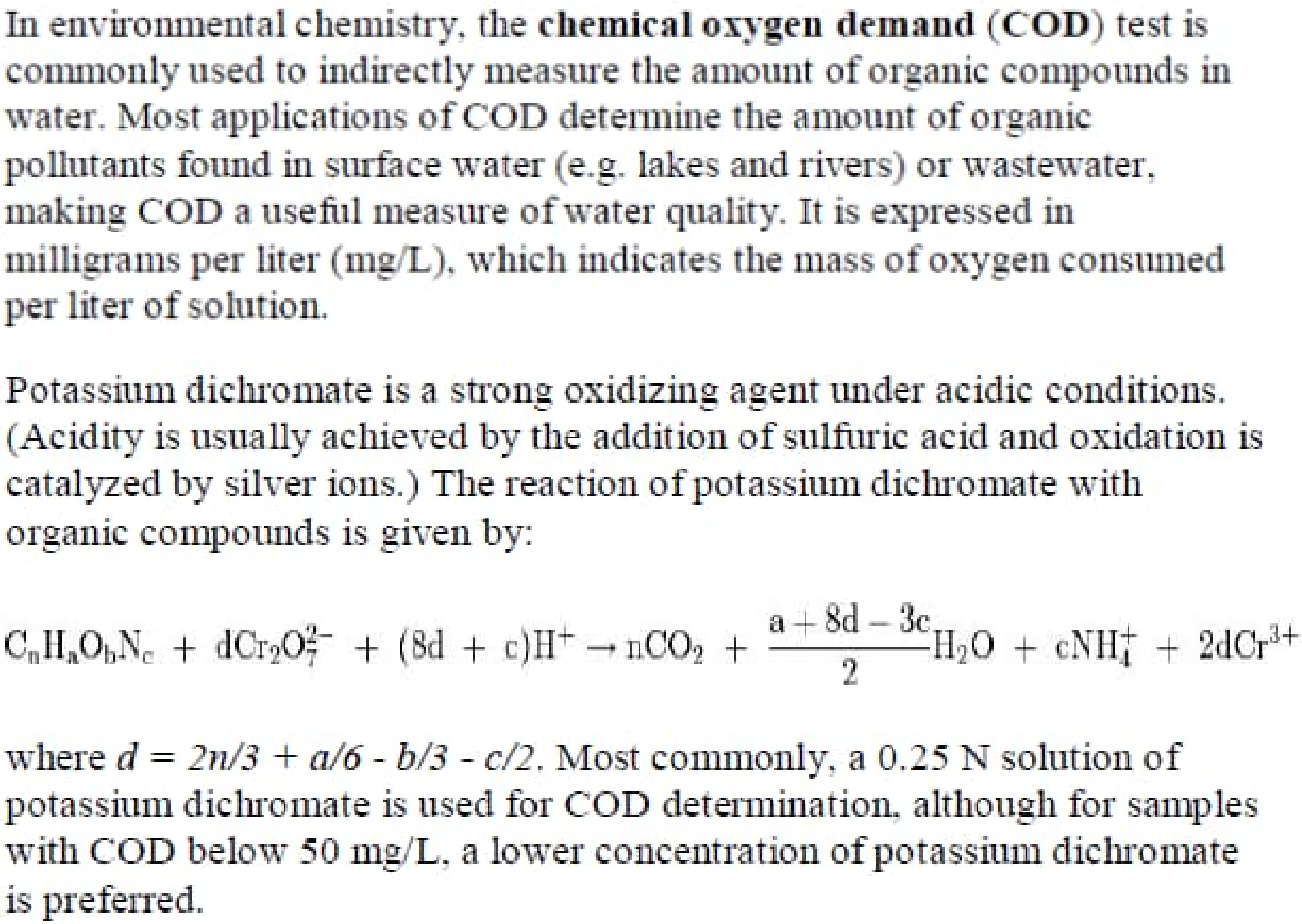
ThOD = $3*2*16$ g-O_2/mol / $(6*1+2*12+16)$ g-Ethanol/mol = 2.8 g-O_2/g-Ethanol

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

Chemical oxygen demand COD



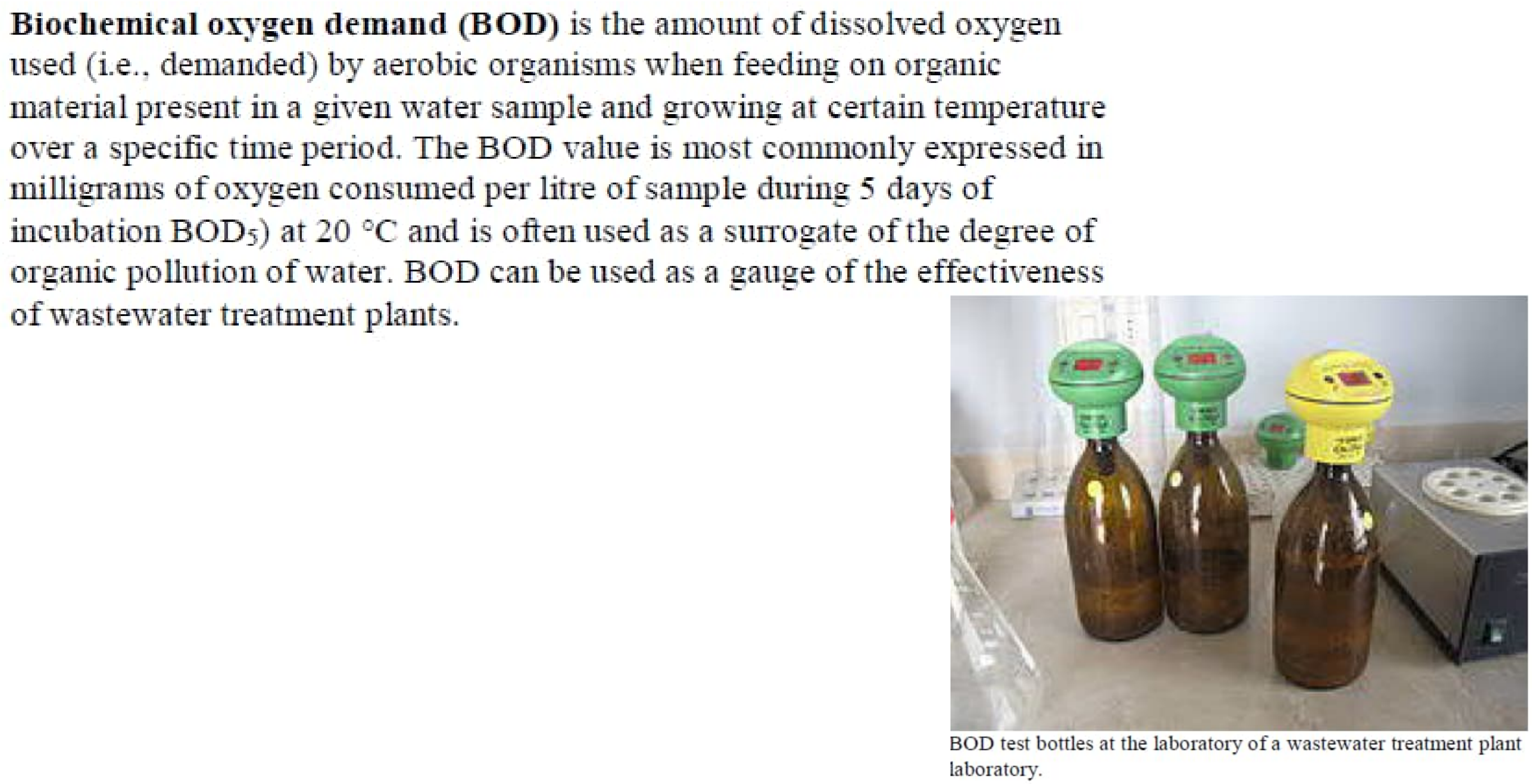
In environmental chemistry, the **chemical oxygen demand** (**COD**) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution.

Potassium dichromate is a strong oxidizing agent under acidic conditions. (Acidity is usually achieved by the addition of sulfuric acid and oxidation is catalyzed by silver ions.) The reaction of potassium dichromate with organic compounds is given by:

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2}H_2O + cNH_4^+ + 2dCr^{3+}$$

where $d = 2n/3 + a/6 - b/3 - c/2$. Most commonly, a 0.25 N solution of potassium dichromate is used for COD determination, although for samples with COD below 50 mg/L, a lower concentration of potassium dichromate is preferred.

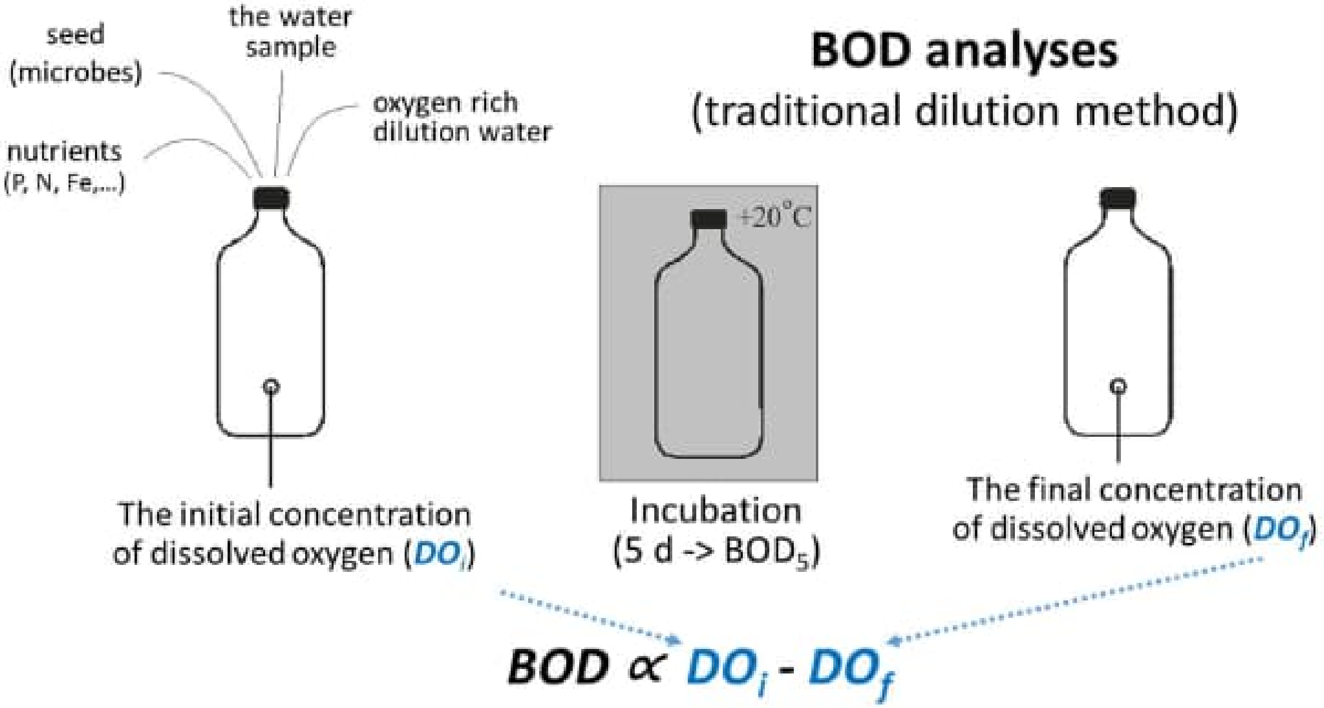
Biological oxygen demand BOD



Biochemical oxygen demand (BOD) is the amount of dissolved oxygen used (i.e., demanded) by aerobic organisms when feeding on organic material present in a given water sample and growing at certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample during 5 days of incubation BOD_5) at 20 °C and is often used as a surrogate of the degree of organic pollution of water. BOD can be used as a gauge of the effectiveness of wastewater treatment plants.

BOD test bottles at the laboratory of a wastewater treatment plant laboratory.

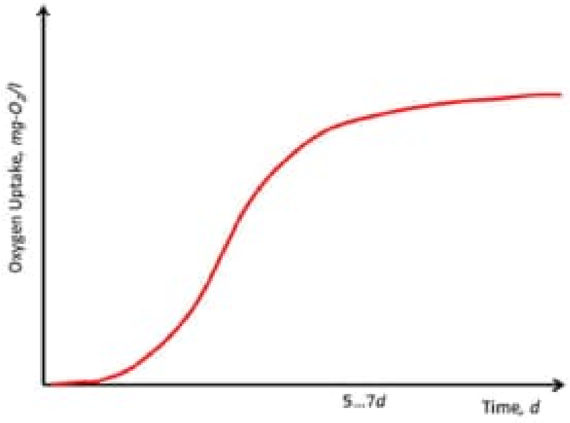
Biological oxygen demand BOD – Dilution method

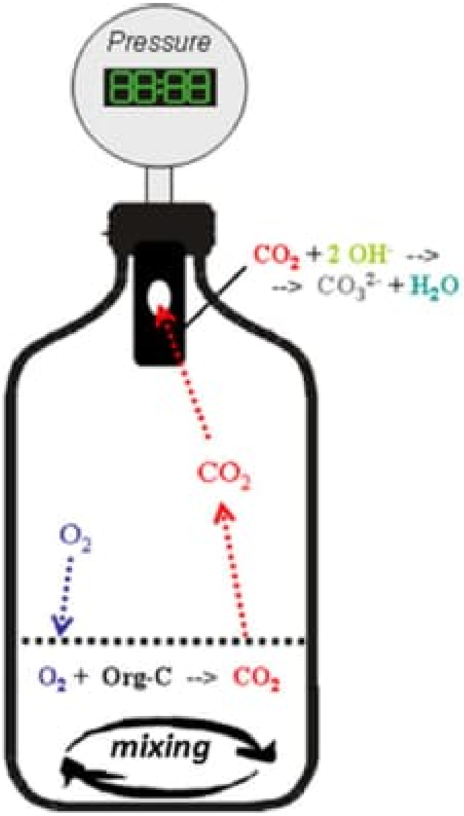


Biological oxygen demand BOD – Manometric method

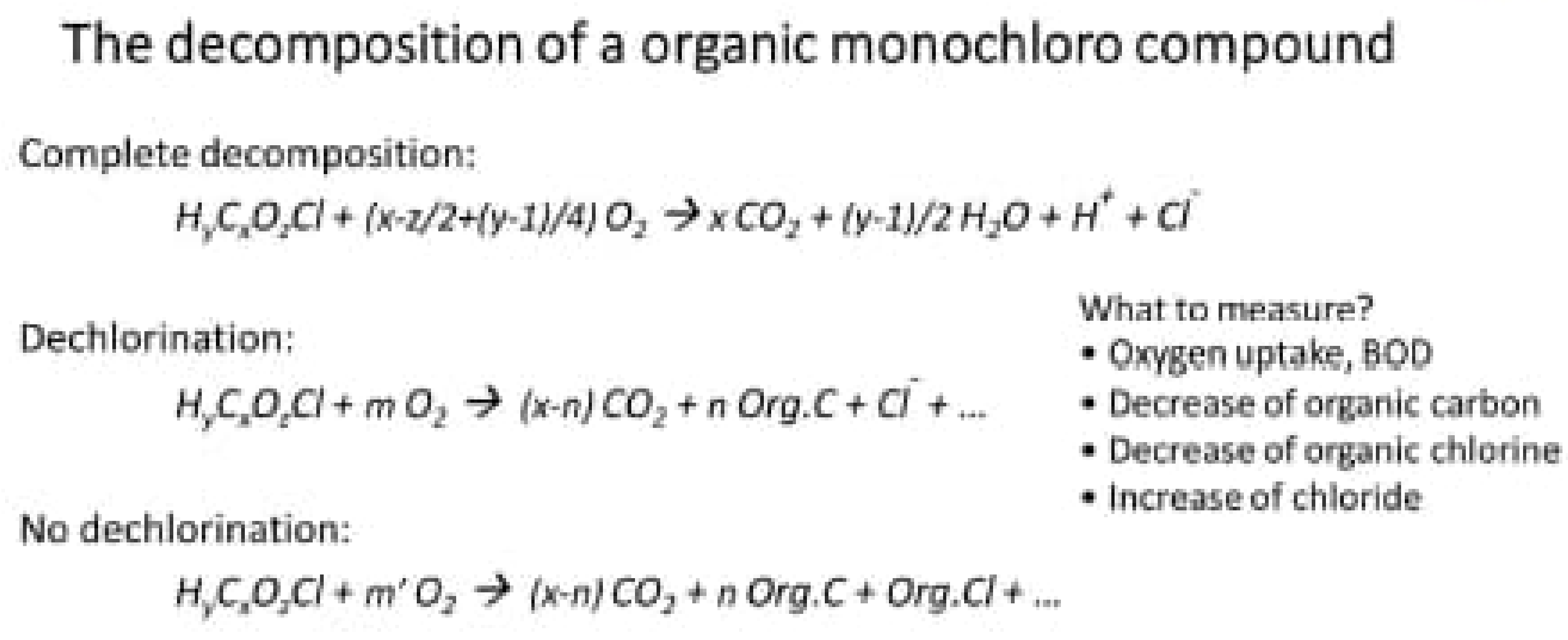
Manometric BOD analyses

BOD ∝ - the change in pressure





Example of a biodegradation process



The decomposition of a organic monochloro compound

Complete decomposition:

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

Dechlorination:

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

No dechlorination:

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

What to measure?

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

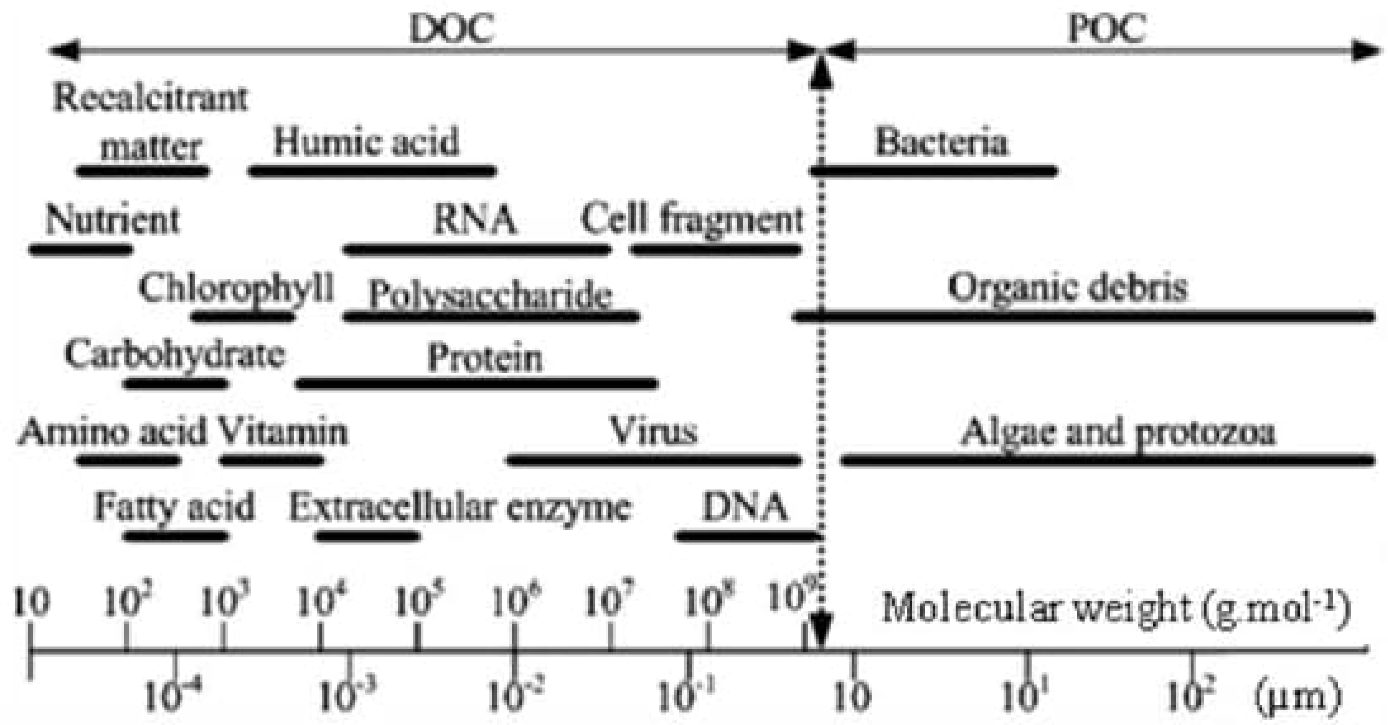
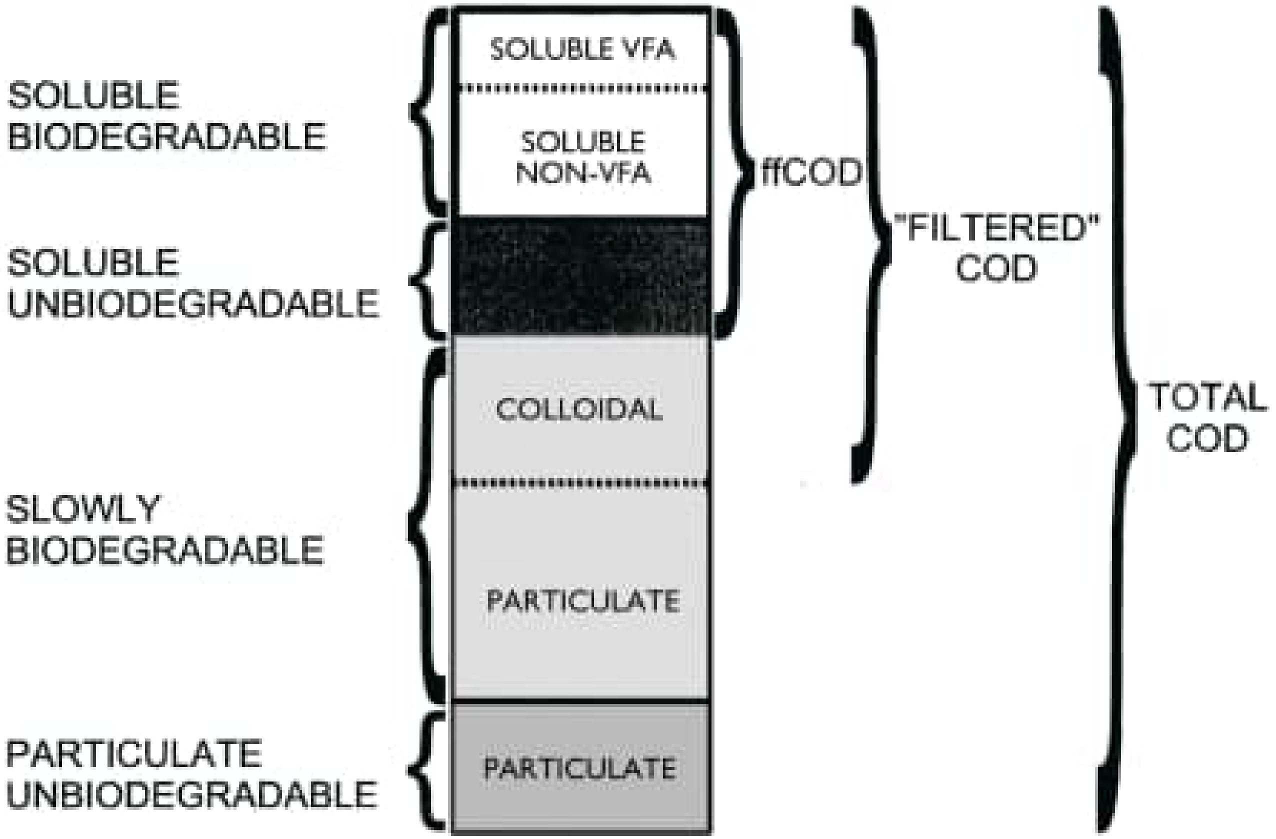


Figure 1: Organic Components in Traditional Wastewater Treatment Plant Effluent. Adapted from Shon et al. (2006).

Fractionation of COD



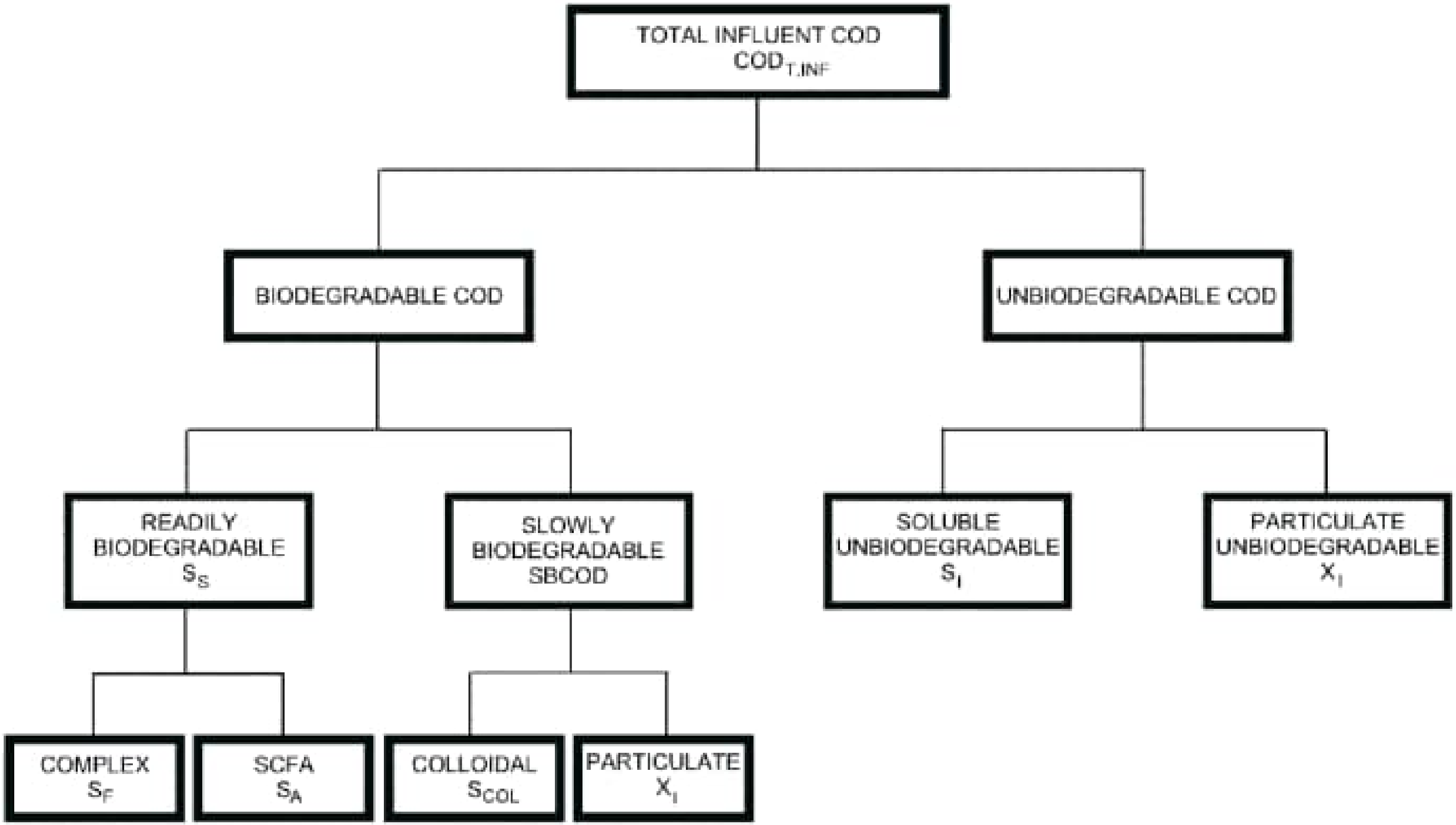
Fractionation of COD – how do the bacteria see it ☺

?

Influent COD fractionation

- **TCOD** $= \underbrace{X_B + X_U}_{\text{Particulate}} + \underbrace{C_B + C_U}_{\text{Colloidal}} + \underbrace{S_B + S_U}_{\text{Soluble}}$
- $\mathbf{FCOD_{1.2\mu m}} = C_B + C_U + S_B + S_U$
- **ffCOD** $= S_B + S_U$ ($ZnSO_4$ at 10 pH using NaOH)
- **RBCOD** $= \text{ffCOD}_{\text{Influent}} - \text{ffCOD}_{\text{Final effluent}}$
- **OUR** for OHO determination

Fractionation of organic matter



TOTAL INFLUENT COD

$COD_{T,INF}$

BIODEGRADABLE COD

UNBIODEGRADABLE COD

READILY BIODEGRADABLE S_S

SLOWLY BIODEGRADABLE SBCOD

SOLUBLE UNBIODEGRADABLE S_I

PARTICULATE UNBIODEGRADABLE X_I

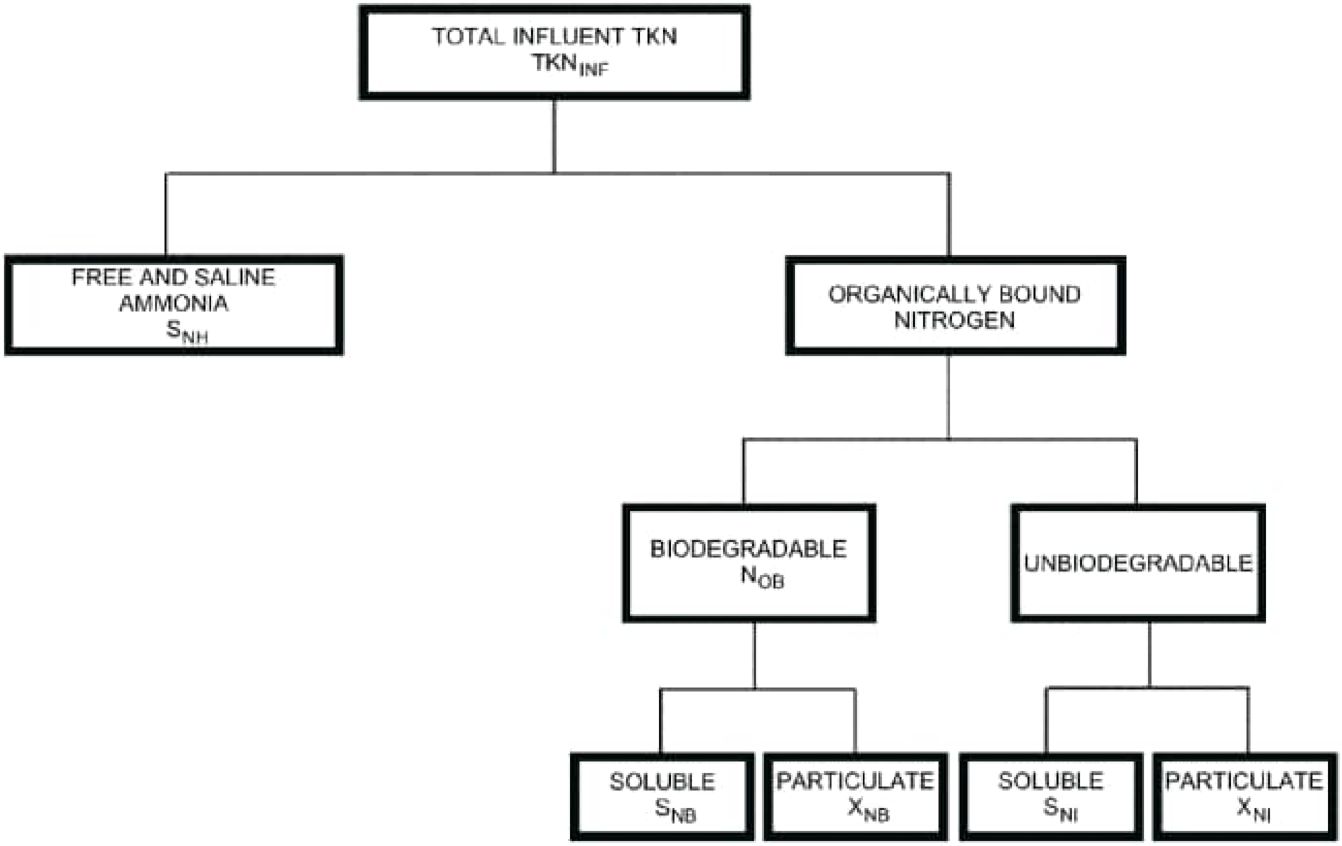
COMPLEX S_F

SCFA S_A

COLLOIDAL S_{COL}

PARTICULATE X_I

Nitrogen



TOTAL INFLUENT TKN
TKN_{INF}

FREE AND SALINE AMMONIA
S_{NH}

ORGANICALLY BOUND NITROGEN

BIODEGRADABLE
N_{OB}

UNBIODEGRADABLE

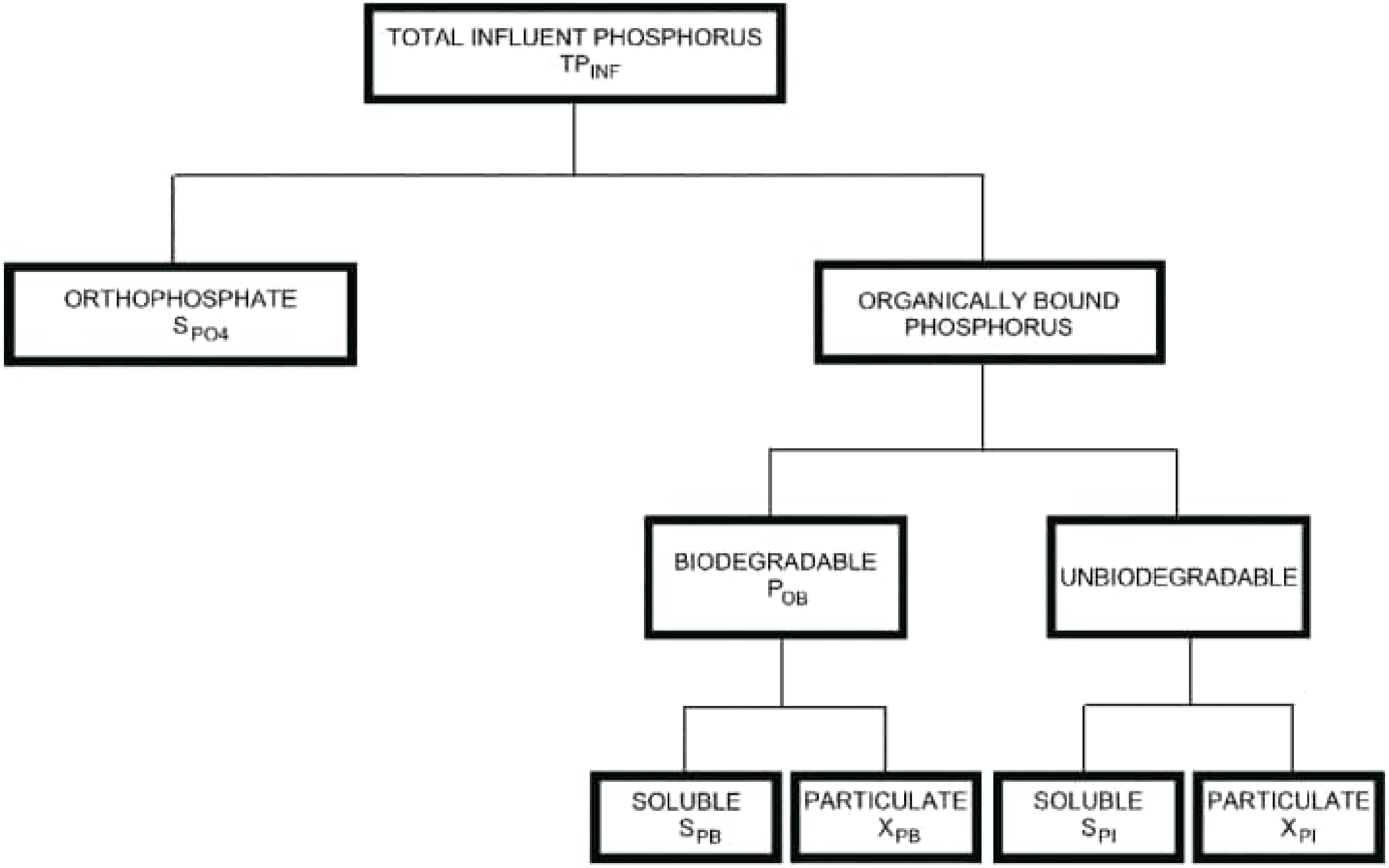
SOLUBLE
S_{NB}

PARTICULATE
X_{NB}

SOLUBLE
S_{NI}

PARTICULATE
X_{NI}

Phophorus

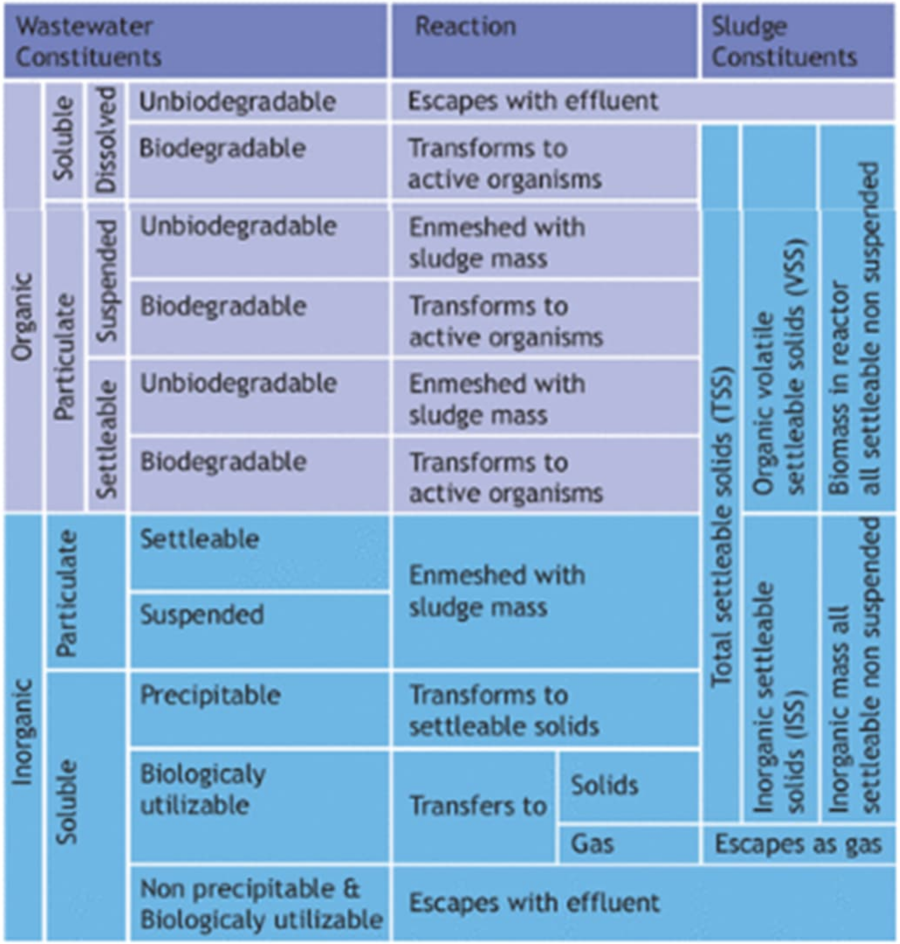

TOTAL INFLUENT PHOSPHORUS
TP_{INF}
ORTHOPHOSPHATE
S_{PO4}
ORGANICALLY BOUND PHOSPHORUS
BIODEGRADABLE
P_{OB}
UNBIODEGRADABLE
SOLUBLE
S_{PB}
PARTICULATE
X_{PB}
SOLUBLE
S_{PI}
PARTICULATE
X_{PI}

Why is influent quality important?

- **Readily biodegradable organic matter**
  - Oxygen demand pattern
  - Determines process's ability to enhanced biological P removal
  - Is denitrification achieved with wastewater's own carbon source
- **Unbioderadable particulate COD**
  - Affects the oxygen demand
  - Affects the sludge yield

- **Influent quality also affects e.g. the performance of the primary clarifier**
- **Biggest differences with industrial wastewater**

Effect of the influent constituents



Wastewater Constituents				Reaction		Sludge Constituents		
Organic	Soluble	Dissolved	Unbiodegradable	Escapes with effluent				
			Biodegradable	Transforms to active organisms		Total settleable solids (TSS)	Organic volatile settleable solids (VSS)	Biomass in reactor all settleable non suspended
	Particulate	Suspended	Unbiodegradable	Enmeshed with sludge mass				
			Biodegradable	Transforms to active organisms				
		Settleable	Unbiodegradable	Enmeshed with sludge mass				
			Biodegradable	Transforms to active organisms				
Inorganic	Particulate		Settleable	Enmeshed with sludge mass			Inorganic settleable solids (ISS)	Inorganic mass all settleable non suspended
			Suspended					
	Soluble		Precipitable	Transforms to settleable solids				
			Biologicaly utilizable	Transfers to	Solids			
					Gas	Escapes as gas		
			Non precipitable & Biologicaly utilizable	Escapes with effluent				

- → **ORGANIC MATTER**
  - → BIODEGRADABLE – forms new biomass
  - → UNBIODEGRADABLE
    - → *PARTICULATE – goes to sludge*
    - → *SOLUBLE – leaves with effluent*
- → **INORGANIC MATTER**
  - → PARTICULATE – goes to sludge
  - → SOLUBLE – precipitable
    - Biologically utilizable
    - The rest escapes with effluent

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

BREAK 10 min

DEMO of COD fractionation

Following tests have been performed with typical municipal wastewater:

Total COD = 913 mg/l

Soluble COD = 657 mg/l

Influent (filtered after flocculation with zinc sulfate) ffCOD = 397 mg/l

Effluent (filtered after flocculation with zinc sulfate) ffCOD = 103 mg/l

In order to estimate the biodegradable organic matter in the sample, a mixture of activated sludge from the plant (V=4,7l) and influent water (V=3,3l) was aerated during 24 hours. Total and soluble COD were measured before and after the aeration. Following results were obtained:

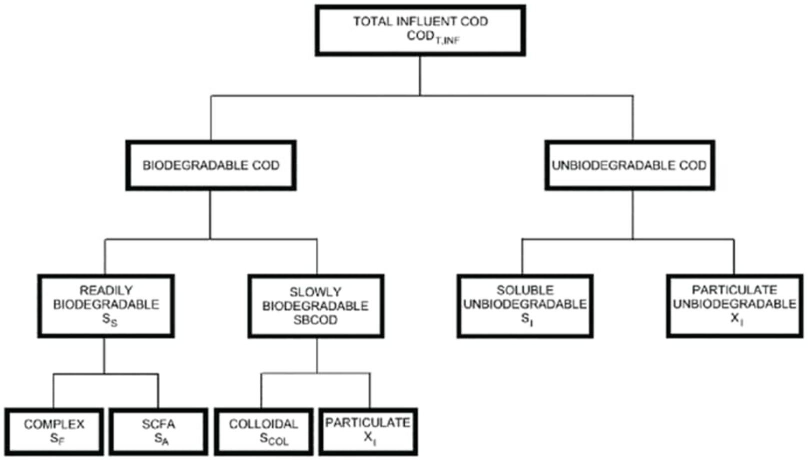
Total COD beginning = 1345 mg/l

Soluble COD beginning = 229 mg/l

Total COD end = 320 mg/l

Soluble COD end = 168 mg/l

NOTE! Zinc sulfate flocculates colloids from the water. Thus, after filtration only soluble COD remains in the water.



DEMO of COD fractionation

Calculate the concentrations and fractions (%) in the influent water of:

readily (RBCOD) and slowly biodegradable(SBCOD) COD

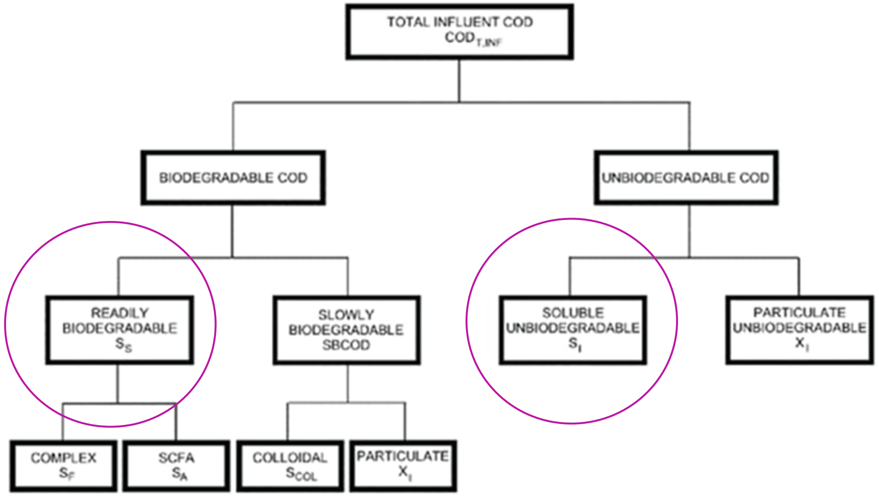
and

soluble and particulate unbiodegradable COD.

From ffCOD we can get the soluble COD fractions (biodegradable and unbiodegradable):

Soluble unbiodegradable = ffCOD effluent = 103 mg/l

Soluble biodegradable = ffCODinf – ffCODeff = 294 mg/l



DEMO of COD fractionation

Calculate the concentrations and fractions (%) in the influent water of:

readily (RBCOD) and slowly biodegradable(SBCOD) COD

and

soluble and particulate unbiodegradable COD.

To be able to continue we will calculate the total biodegradable COD from the 24 h aeration test:

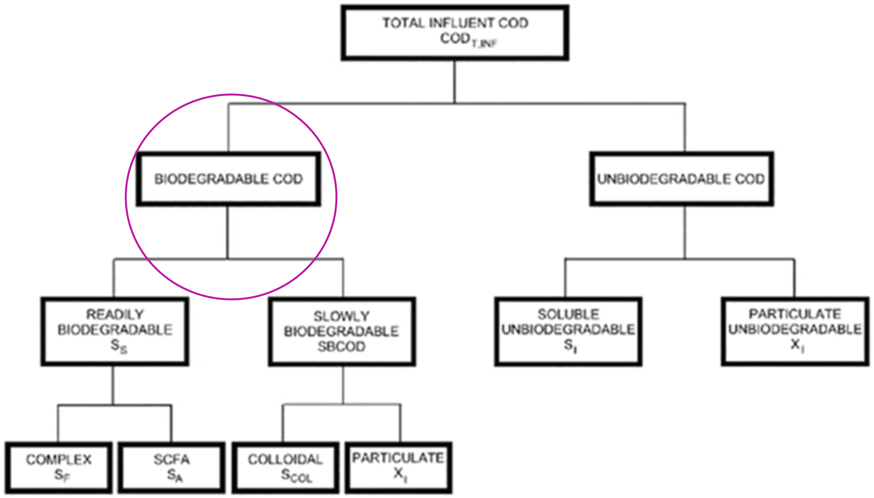
Particulate COD in the WW: CODtot – CODsol = 256 mg/l

Particulate COD after addition of sludge = 1116 mg/l → COD of added sludge (in the mixture) = 860 mg/l

Then we can calculate the CODinfluent in the mixture = 1345 – 860 = 485 mg/l

Of this 168 mg/l is unbiodegradable (=soluble COD after 24 h aeration) → biodegradable (in the mixture) = 485 – 168 = 317 mg/l

Taking into account the addition of sludge: dilution of 2,4 (from 3,3 l to 8 l) → Total biodegradable 768 mg/l



DEMO of COD fractionation

Calculate the concentrations and fractions (%) in the influent water of:

readily (RBCOD) and slowly biodegradable(SBCOD) COD

and

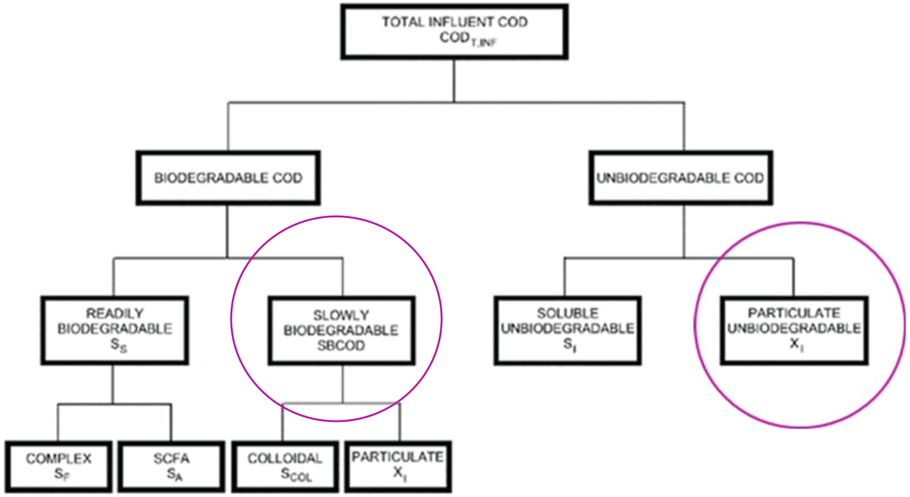
soluble and particulate unbiodegradable COD.

Then we will calculate the SBCOD and particulate unbiodegradable:

SBCOD = Total biodegradable – RBCOD = 768 – 294 = 474 mg/l

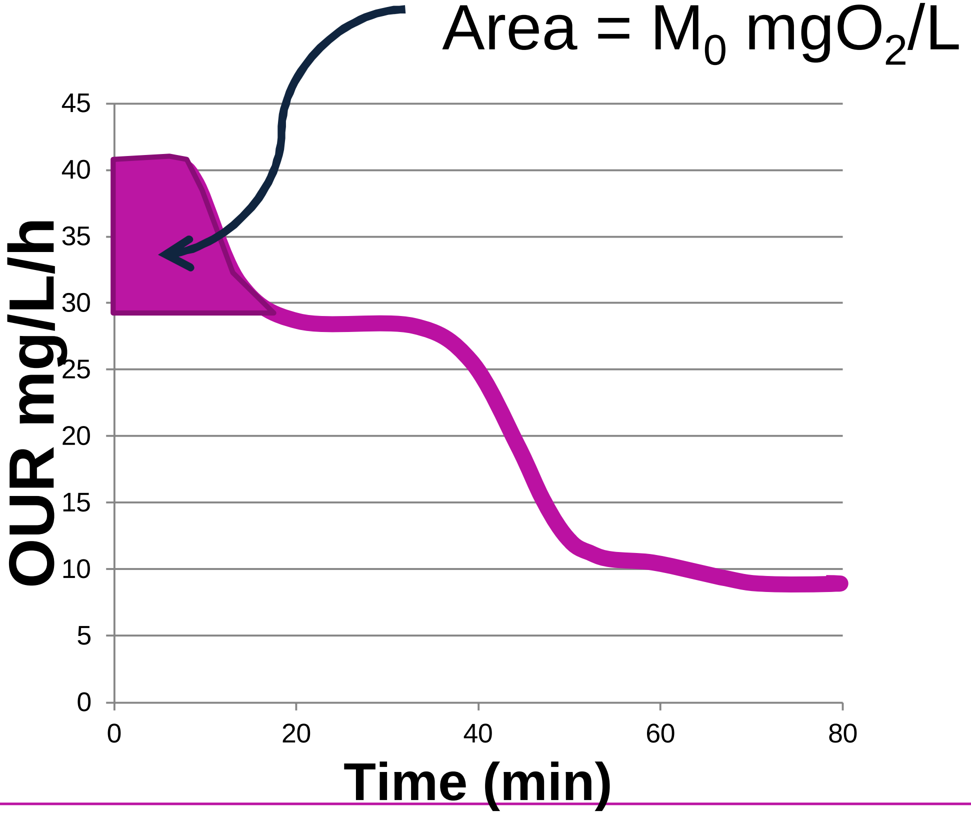
Total unbiodegradable = Total COD – biodegradable = 913 – 768 = 144 mg/l

And finally particulate unbiodegradable = Total unbiodegradable – soluble unbiodegradable = 144 – 103 = 41 mg/l

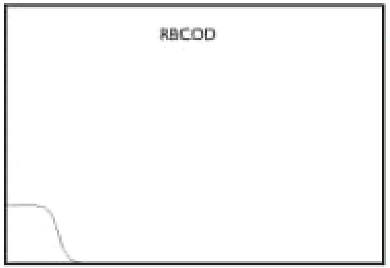


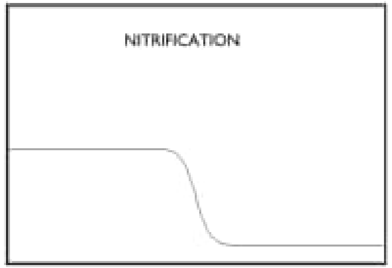
Alternative for RBCOD: RBCOD measurement by respirometry

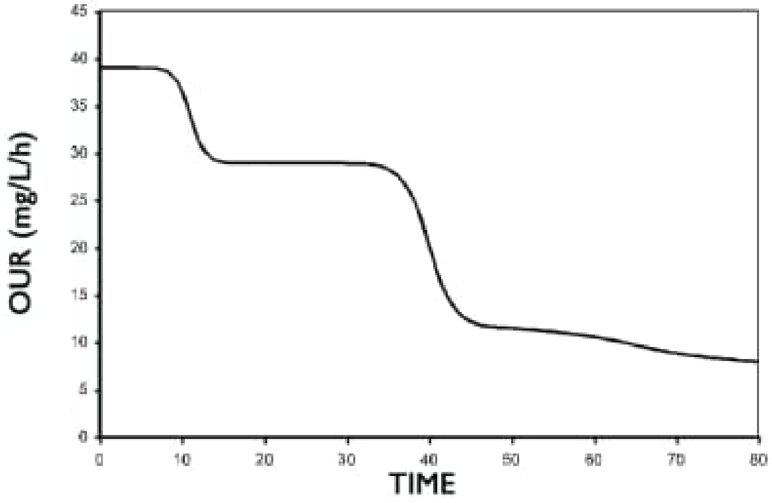
Respirogram

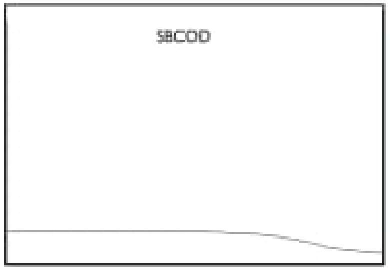


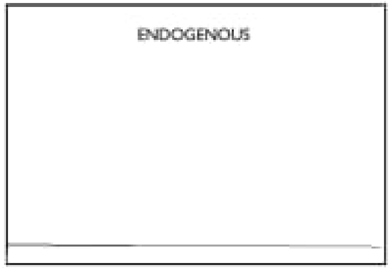
Components contributing to measured OUR











Introduction to biological processes

Metabolism of living cells

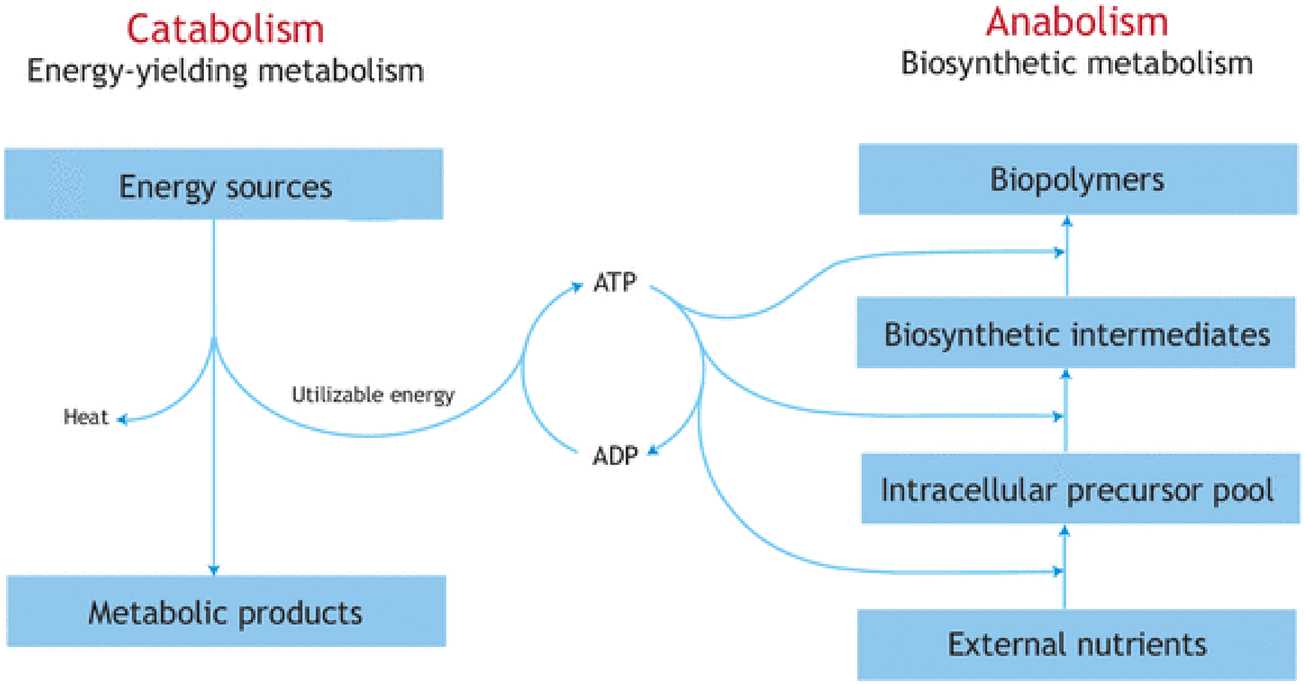
Catabolism = energy supply of the cell (redox reaction)

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

Main requirements

→ Electron donor and acceptor

→ Carbon source



How do bacteria obtain energy?

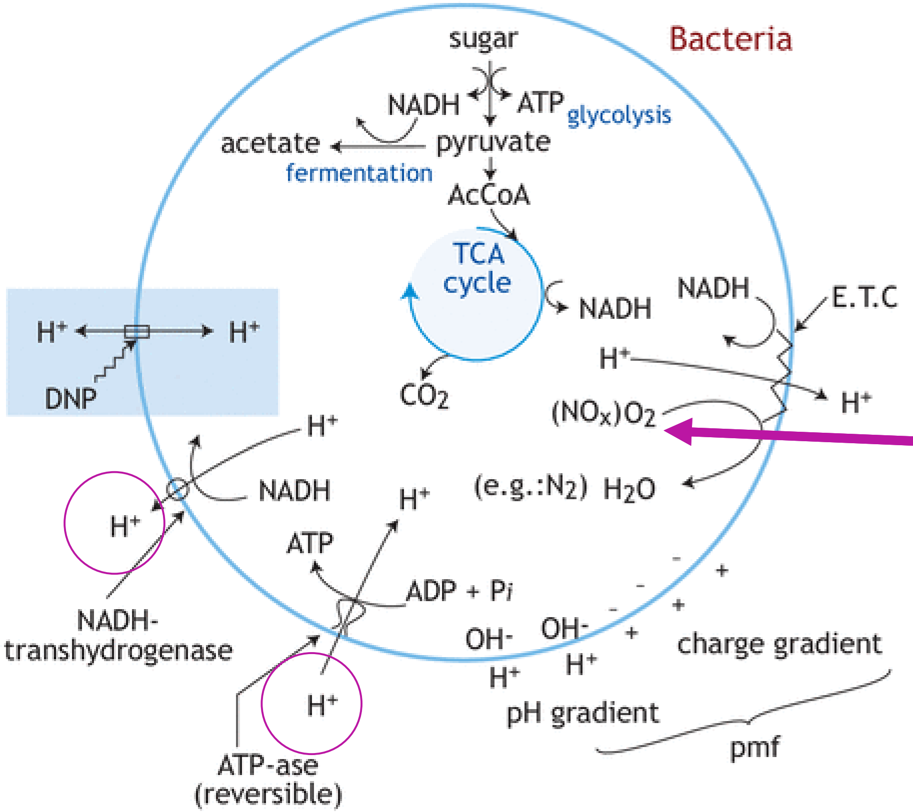


Figure 2.13 Overview of bacterial bioenergetics (adapted from Comeau *et al.*, 1986)

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=ubzw64PQPqM

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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

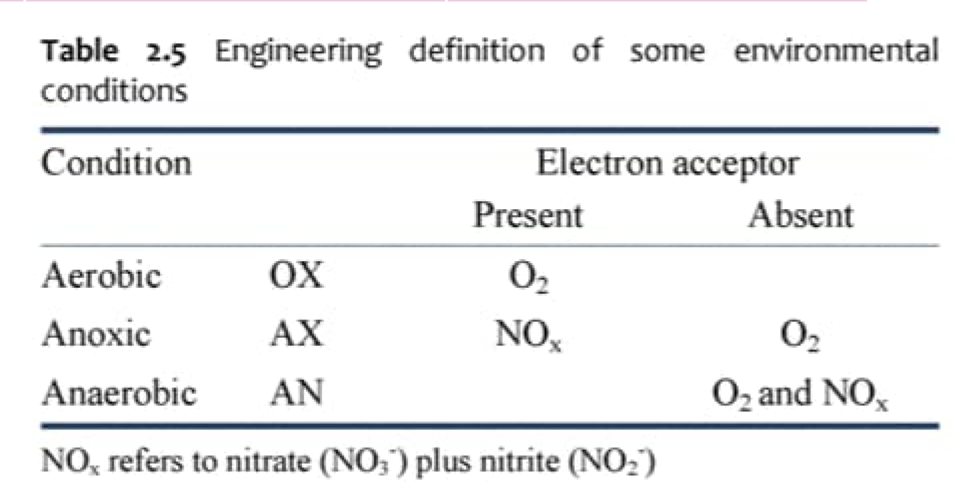


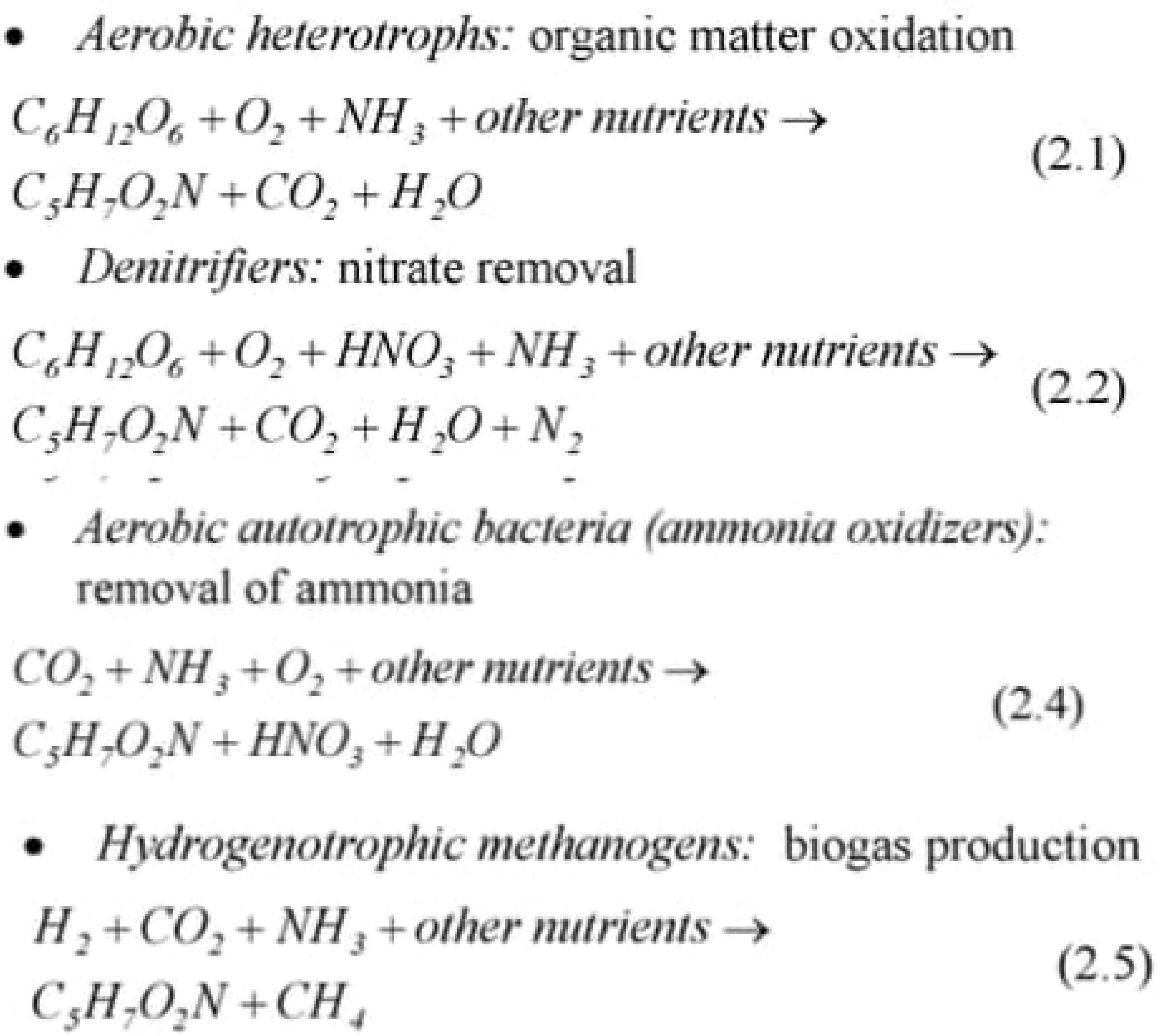
Table 2.5 Engineering definition of some environmental conditions

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^-)

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

Examples of reactions



- *Aerobic heterotrophs:* organic matter oxidation

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

- *Denitrifiers:* nitrate removal

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

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

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

- *Hydrogenotrophic methanogens:* biogas production

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

Microorganismsin water and wastewater treatment

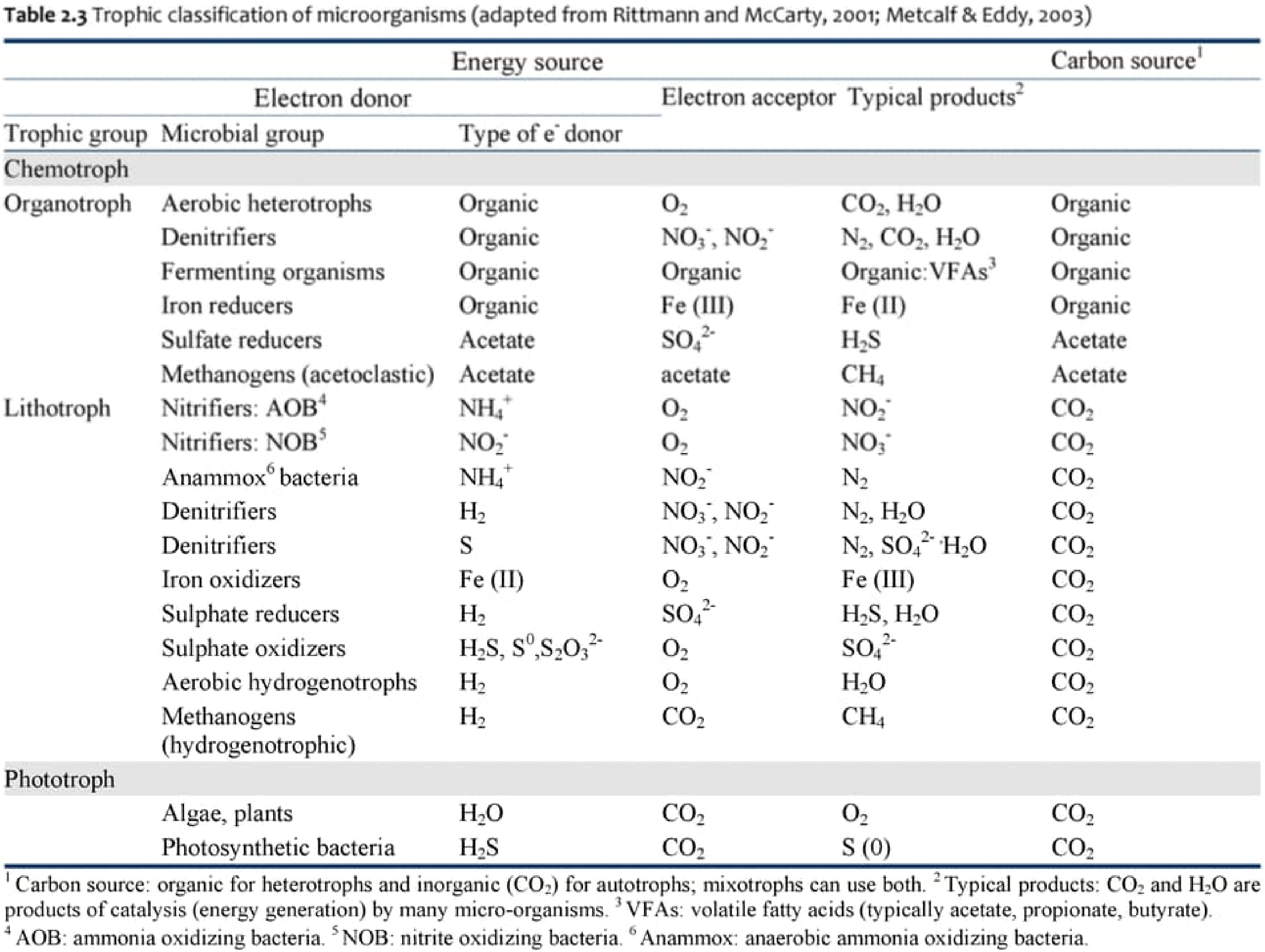


Table 2.3 Trophic classification of microorganisms (adapted from Rittmann and McCarty, 2001; Metcalf & Eddy, 2003)

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.

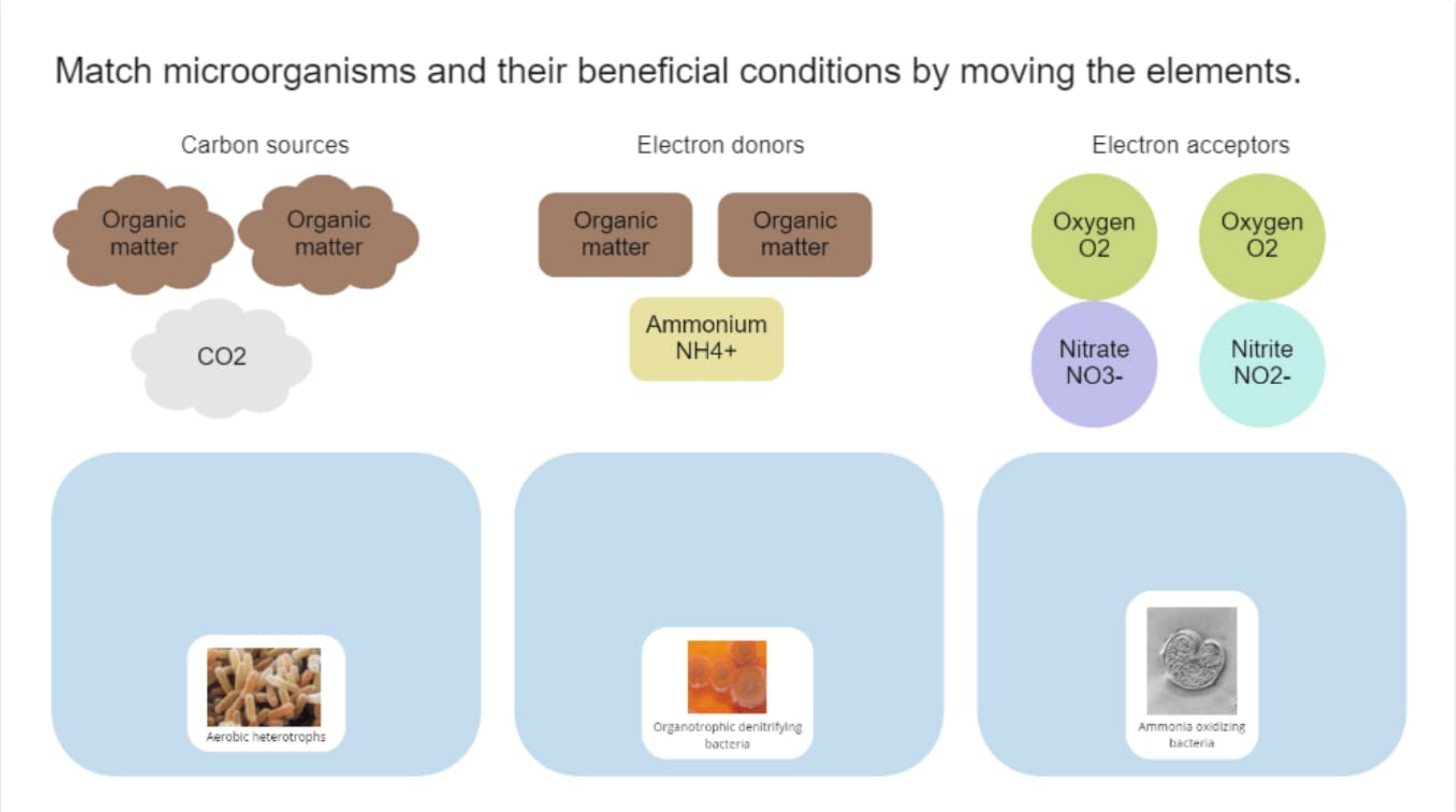
Ready for BACTERIA GAME?

- Three volunteers needed for bacteria
- Others will receive cards with different chemical components
- Go around and discuss to find suitable components for each bacteria

BACTERIA GAME ALSO IN MIRO?

- You can also rehearse alone in Miro
- You can find the link in MyCourses
- Move the carbon sources and electron donors and acceptors in the game in order to create beneficial conditions for each bacteria

Game start



Match microorganisms and their beneficial conditions by moving the elements.

Carbon sources

Organic matter

Organic matter

CO_2

Electron donors

Organic matter

Organic matter

Ammonium NH_4^+

Electron acceptors

Oxygen O_2

Oxygen O_2

Nitrate NO_3^-

Nitrite NO_2^-

Aerobic heterotrophs

Organotrophic denitrifying bacteria

Ammonia oxidizing bacteria

Reading material

Biological wastewater treatment:

Chapters

3.3 – 3.14

10.1 – 10.2 + 10.5