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**SWIM and Horizon 2020 SM REG-14: Refugee Emergency: Fast track project Design of wastewater**

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SESSION -1 PART 2
MICROORGANISMS
1. Typical Microorganisms in Wastewater
2. Classification of Bacteria
3. Bacteria Metabolism
4. Bacteria growth
5. Yield
6. Growth kinetic for Nitrification
7. Activated sludge kinetic coefficients for Heterotrophic bacteria
8. Activated sludge kinetic coefficients for Nitrifying bacteria
PURPOSE OF WASTEWATER TREATMENT

IS TO...

Transform

Particulate & Dissolved Organics

INTO

Biomass (Primarily Bacterial Bodies)

Why?

Because Bacteria Settle & Dissolved Solids Don’t
If we have 1 unit of COD substrate 1 mg COD/l (let's say that it is totally biodegradable), then we will grow $Y$ units of cell mass, and $1-Y$ will be oxidized for energy.
TYPICAL MICROORGANISMS IN WASTEWATER

- Microorganisms
  - Bacteria
  - Fungi Yeast
  - Protozoa
  - Rotifers
  - Algae
  - Viruses
## Classification of Bacteria Based on Nutritional Requirements

<table>
<thead>
<tr>
<th>Item</th>
<th>Heterotrophic Bacteria</th>
<th>Autotrophic Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Source</td>
<td>Organic compounds</td>
<td>Inorganic Compounds</td>
</tr>
<tr>
<td>Carbon Source</td>
<td>Organic compounds</td>
<td>CO₂</td>
</tr>
<tr>
<td>Types</td>
<td>Aerobic Bacteria</td>
<td>Nitrifying Bacteria</td>
</tr>
<tr>
<td></td>
<td>Anaerobic Bacteria</td>
<td>Nitrosomonas</td>
</tr>
<tr>
<td></td>
<td>Facultative Bacteria</td>
<td>Nitrobacter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulfur Bacteria</td>
</tr>
</tbody>
</table>
METABOLISM OF HETEROTROPHIC BACTERIA

- Organic matter is the substrate (food) used as energy source.
- The majority of organic matter in wastewater is in the form of large molecules which can’t penetrate the bacteria cell membrane.
- Therefore large molecules are **hydrolyzed** into diffusible reactions for assimilation into their cells. The first biochemical reactions are **hydrolysis** of:
  - Complex carbohydrates → sugar units
  - Proteins → amino acids
  - Insoluble fats → fatty acids
- Under aerobic conditions the reduced soluble organics compounds are oxidized to end products of CO2 and water.

\[
\text{Organics (Aerobic)} + O_2 \rightarrow CO_2 + H_2 O + \text{energy}
\]

- Under anaerobic conditions, soluble organics are decomposed to intermediate end products (H2S, organic acids) along with production of CO2 water.

\[
\text{Organics (Anaerobic)} \rightarrow \text{Intermediates} + CO_2 + H_2 O + \text{energy}
\]
METABOLISM OF AUTOTROPHIC BACTERIA

- Autotrophic bacteria use CO2 as a carbon source and oxidize inorganic compounds for energy.
- **Nitrifying Bacteria**
  
  \[ \text{NH}_3 + O_2 \xrightarrow{\text{Nitrosomonas}} \text{NO}_2^- + \text{energy} \]

  \[ \text{NO}_2^- + O_2 \xrightarrow{\text{Nitrobacter}} \text{NO}_3^- + \text{energy} \]

- **Sulfur Bacteria**

  \[ \text{H}_2\text{S} + O_2 \rightarrow \text{H}_2\text{SO}_4 + \text{energy} \]

- **Iron Bacteria**

  \[ \text{Fe}^{2+} + O_2 \rightarrow \text{Fe}^{3+} + \text{energy} \]
BACTERIAL GROWTH PATTERNS

- Log Growth Phase
- Declining Growth
- Endogenous Phase / Death Phase

High Rate

Conv. Rate

Microorganism Mass

Extended Aeration

Organic Food (BOD5)

TIME

MASS

High Rate

Conv. Rate

Extended Aeration

Organic Food (BOD5)
HETEROTROPHIC BACTERIAL GROWTH

To Grow Bacteria
- Make Food Available
- Make Oxygen Available
- Make Nutrients Available
- Provide Warm Temperature

Bacteria
(Growth & Reproduction)

Soluble Organics

Nitrogen & Phosphorus

Carbon Dioxide

Oxygen

HETEROTROPHIC BACTERIAL GROWTH

Soluble Organics

Nitrogen & Phosphorus

Carbon Dioxide

Oxygen

Bacteria
(Growth & Reproduction)

To Grow Bacteria
- Make Food Available
- Make Oxygen Available
- Make Nutrients Available
- Provide Warm Temperature
Nitrifying Bacteria
(Growth & Reproduction)

To Grow Nitrifiers
- Make Food Available
- Make Oxygen Available
- Provide Warm Temperature
- Prevent Low pH
DEFINITIONS & TERMINOLOGY

• **Substrate**
  – Carbon source (organic and inorganic)

• **Heterotrophs**
  – Organisms that use organic carbon to produce new cells.
    • e.g. most organisms used in aerobic treatment of wastewater

• **Autotrophs**
  – Organisms that use CO2 to produce new cells.
    • e.g. Nitrifying bacteria

• **Nutrients**
  – Essential For Growth and Maintenance Of Micro-organisms
  • e.g. Macro nutrients (N and P). Micro nutrients (Metals such as Fe, Ca, Mg, K, Mo, Zn, Co)
Definitions & Terminology

• **Mixed Liquor (ML) & Mixed Liquor Suspended Solids (MLSS)**
  - ML, Mixture of wastewater and microorganisms (bugs) in the aeration tank.
  - MLSS, Concentration of bugs in the mixed liquor.

• **Microorganisms (Bugs)**
  - Microscopic living objects which require energy, carbon and small amount of inorganic elements to grow and multiply. They get these requirements from the wastewater and the sun and in doing so help to remove the pollutant from wastewater.

• **Nitrification**
  - Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed with the oxidation of these nitrites into nitrates.

• **Denitrification**
  - The reduction of nitrate or nitrite to gaseous products such as nitrogen, nitrous oxide, and nitric oxide; brought about by denitrifying bacteria.

• **Lysis**
  - To separate, breakdown of a cell often by viral, enzymic or osmotic by rupture of the cells wall.
MICROBIAL GROWTH KINETICS
The metabolic activity of bacteria is the primary means of removing pollutants in a bioreactor. Estimating production of biomass is a key step in the analysis and design of activated sludge system in general and bioreactor in particular and involve two aspects:

- **Kinetics**, which deals with rates of bacterial growth and decay reactions (how fast the reactions will occur)
- **Stoichiometry**, which deals with reactions and relationships between the masses of reactants and products involved in the reactions.

Reactions affecting biomass production are:
- Bacterial growth from uptake of substrate.
- Bacterial loss from decay or endogenous respiration.
Synthesis or true yield \( (Y_\text{t}) \) is a stoichiometric parameter that is generally defined as the mass of biomass produced per unit mass of biodegradable substrate consumed. This represents the “true yield”, applies only to the biodegradable fraction of the substrate and active biomass produced, and doesn’t account for the effect of biomass decay.

\[
Y = \frac{\text{Biomass}}{\text{Substrate}} = \frac{\mu_{\text{max}}}{k}
\]

Where
- \( Y \) = synthesis or true yield.
- \( k \) = maximum specific substrate utilization rate.
- \( \mu_{\text{max}} \) = maximum specific growth rate, 1/d.
The net biomass yield ($Y_{net}$ or $Y_{bio}$) is different than the “true yield”, it is defined as the ratio of net biomass growth rate to the substrate utilization rate. It includes biomass decay. It is used to estimate the amount of active microorganisms in the system.

\[
Y_{net} = \frac{\text{Net}_\text{bio}_\text{mass}_\text{production}}{\text{substrate}_\text{utilization}_\text{rate}}
\]

\[
Y_{net} = \frac{\text{production} - \text{decay}}{\text{substrate}_\text{utilization}_\text{rate}}
\]

\[
Y_{net} = \frac{Y}{1 + k_d \times SRT}
\]

Where

$Y_{net}$ = net biomass yield, g biomass (AVSS)/ g substrate used.
The observed yield $Y_{obs}$ is based on the actual measurement of the amount of solids production relative to the substrate removal.

$$Y_{obs} = \frac{\text{Total}_\text{MLVSS}_\text{Production}_\text{Rate}}{\text{Substrate}_\text{removal}_\text{Rate}}$$

$$Y_{obs} = \frac{P_{X,VSS}}{Q(S_o - S)}$$

$$Y_{obs} = \frac{P_{X,TSS}}{Q(S_o - S)}$$

Where

$Y_{obs}$ = observed yield, g VSS produced/g substrate removed.
g TSS produced/g substrate removed.

$P_{X,VSS}$ = net waste activated sludge produced, kg VSS/day.

$P_{X,TSS}$ = net waste activated sludge produced, kg TSS/day.

$Q$ = Average influent flow

$S_0$ = influent substrate concentration (mg/l)

$S$ = effluent substrate concentration (mg/l)
STOICHIOMETRY

• Most simulation models base all calculations of organic material (including biomass) on COD.
• For practical purposes biomass has to be expressed as suspended solids.
• It is estimated that 1 gram of volatile suspended solids is equal to 1.42 gram of COD. This is based on the assumption that the composition of a typical bacterial cell (biomass) can be characterized as $C_5H_7NO_2$.

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3$$

• This implies that 160 g of oxygen are required (COD) to completely oxidize 113 g of biomass (VSS). Thus the COD/VSS ratio is $160/113=1.42$. 
HALF-VELOCITY CONSTANT \((K_s)\)

- The half-velocity constant is the value of the soluble substrate concentration at an one-half the maximum specific substrate utilization rate.

- The relationship developed by Monod(1949) forms basis for bacterial growth kinetics.
- Rate of bacterial growth and substrate utilization is a function of the concentration of the limiting substrate (e.g. BOD or NH3) surrounding the bacteria and the concentration of active bacteria.
\[ k_T = k_{20} \times \theta^{(T-20)} \]

Where
- \( k_T \) = reaction rate coefficient at temperature \( T, ^\circ C \).
- \( k_{20} \) = reaction rate coefficient at 20 \( ^\circ C \).
- \( \theta \) = temperature coefficient (1.02-1.25).
- \( T \) = temperature, \( ^\circ C \).
The effluent soluble substrate concentration for a complete mix activated sludge process is only a function of the SRT and kinetic coefficients for growth and decay.

The effluent substrate concentration is not related to the influent soluble substrate concentration.

The influent concentration affects the biomass concentration.

\[
S = \frac{K_S \left[ 1 + k_d SRT \right]}{SRT (Yk - k_d) - 1}
\]

\[
\mu_m = kY
\]

Where:
- \( S \): effluent soluble substrate concentration g BOD or bsCOD/m3
- \( SRT \): sludge age
- \( \mu_m \): maximum specific growth rate, 1/d.
- \( K_S \): Half-velocity constant
- \( k_d \): endogenous decay coefficient, g VSS/gVSS.d
- \( Y \): True yield heterotrophs.
- \( k \): Maximum specific substrate utilization rate
The reactor biomass concentration is function of:

- SRT
- Aerobic detention time.
- Yield coefficient
- Amount of substrate removed \((S-S_o)\).

**Where:**

- \(X\) : Biomass concentration, g/m³
- SRT : sludge age
- \(k_d\) : endogenous decay coefficient, g VSS/gVSS.d
- \(Y\) : True yield heterotrophs, mg VSS/mg BOD or COD
- \(S_o\) : Influent soluble substrate concentration g BOD or bsCOD/m³
- \(S\) : Effluent soluble substrate concentration, BOD or bsCOD/m³.
- \(\tau\) : hydraulic detention time, V/Q, day.

\[
X = \left(\frac{SRT}{\tau}\right) \left[\frac{Y(S_o - S)}{1 + k_d SRT}\right]
\]

\[
X = \left(\frac{SRT}{V}\right) \left[\frac{YQ(S_o - S)}{1 + k_d SRT}\right]
\]
DECAY (LOSS OF BIOMASS)
CELL DEBRIS (ENDOGENOUS PRODUCTS)

- VSS in the bioreactors include:
  - Active biomass.
  - nbVSS in the influent wastewater.
  - Cell debris.
- Cell debris
  - The remaining non-biodegradable material after cell death and lysis.
  - Cell wall is non-biodegradable and contribute to the cell debris.
  - Cell debris represents about 10 to 15 percent of the original cell weight.
- The rate of production of cell debris is directly proportional to the endogenous decay rate.

\[ r_{XD} = f_d \times k_d \times X \]

Where:
- \( r_{XD} \): rate of cell debris production, g VSS/m3.d
- \( f_d \): fraction of biomass that remains as cell debris (0.10-0.15 g VSS/g VSS)
- \( k_d \): endogenous decay rate, g VSS/gVSS.d
- \( X \): Active biomass concentration mg/l
Total Volatile suspended solids = Biomass production
  + nbVSS from cell debris
  + nbVSS in influent
ACTIVE BIOMASS (AVSS) FRACTION

\[ F_{AVSS} = \frac{\text{Net\_Biomass\_Production}(\text{growth} - \text{decay})}{\text{MLVSS}} \]
MODELLING SUSPENDED GROWTH TREATMENT PROCESSES
BIOMASS MASS BALANCE

Rate of accumulation of microorganisms within the system

Rate of flow of microorganisms into the system

Rate of flow of microorganisms out of the system

Net growth of microorganisms

Influent $Q, S_0, X_0$

Aeration Tank $S, X, V$

Clarifier Effluent $(Q-Q_w)$ $X_e, S_o$

Return Activated Sludge $Q_r, S_r, S$

Sludge $Q_w, X_r, S$
GROWTH KINETICS FOR NITRIFICATIONS

\[
\mu_n = \mu_{nm} \times \frac{N}{K_N + N} - k_{dn}
\]

Assuming excess DO is available

\[
\mu_n = \mu_{nm} \times \frac{N}{K_N + N} \times \frac{DO}{K_O + DO} - k_{dn}
\]

Accounts for DO concentration

Where

- \(\mu_n\) = specific growth rate of nitrifying bacteria, g new cells/g cells.d
- \(\mu_{nm}\) = maximum specific growth rate of nitrifying bacteria, g new cells/g cells.d
- \(N\) = nitrogen concentration, g/m3.
- \(K_n\) = half-velocity constant for ammonia concentration, substrate concentration at one half the maximum specific substrate utilization rate, g/m3.
- \(k_{dn}\) = endogenous decay coefficient for nitrifying organisms, g VSS/g VSS.d.
- \(DO\) = DO concentration
- \(K_O\) = half-velocity constant for DO concentration
For further information

Website
www.swim-h2020.eu  E: info@swim-h2020.eu

LinkedIn Page
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Facebook Page
SWIM-H2020 SM Facebook
SWIM and Horizon 2020 Support Mechanism
Working for a Sustainable Mediterranean, Caring for our Future

Thank you for your attention.
Appendix
MAXIMUM RATE OF SOLUBLE SUBSTRATE UTILIZATION ($k$)

- This coefficient is used for designing a complete-mix activated-sludge system and has to do with the biomass growth process. Substrate in biological processes used for wastewater treatment, referers to the organic matter or nutrients in wastewater, that are converted during biological treatment or that may be limiting in biological treatment.

- In specific circumstances, there will be a certain rate with which the soluble substrate will be depleted by bacteria. At a high substrate concentration, the utilization rate will be high and will be practically even to the maximum rate of soluble substrate utilization.

\[ \mu_m = kY \]
<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Unit</th>
<th>Value @ 20 °C</th>
<th>Temp. Correction (θ Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum specific bacterial growth rate</td>
<td>μm</td>
<td>3-13.2</td>
<td>1.03-1.08, 1.07</td>
</tr>
<tr>
<td>Half-velocity constant</td>
<td>Ks</td>
<td>10-60</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>mg bsCOD/l</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>mg bCOD/l</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>True yield /Synthesis yield coefficient</td>
<td>Y</td>
<td>0.4-0.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>mgVSS/mg BOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mgVSS/mg bCOD</td>
<td>0.3-0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Endogenous decay coefficient</td>
<td>k_d</td>
<td>0.06-0.2</td>
<td>1.03-1.08, 1.04</td>
</tr>
<tr>
<td></td>
<td>g VSS/g VSS.day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell debris fraction</td>
<td>f_d</td>
<td>0.08-0.2</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Source: Tale 8-10, Metcalf & Eddy, page 705.
## COMPARISON OF TYPICAL KINETIC COEFFICIENTS FOR AEROBIC OXIDATION OF BOD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Metcalf &amp; Eddy/AECOM Fifth Edition</th>
<th>Metcalf &amp; Eddy Fourth Edition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Typical</td>
</tr>
<tr>
<td>Maximum specific substrate</td>
<td>k</td>
<td>4-12</td>
<td>6</td>
</tr>
<tr>
<td>utilization rate</td>
<td>bsCOD/g VSS.d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-velocity constant</td>
<td>Ks</td>
<td>20-60</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>mg/l BOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/l bsCOD</td>
<td>5-30</td>
<td>15</td>
</tr>
<tr>
<td>True yield /Synthesis yield</td>
<td>Y</td>
<td>0.4-0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>coefficient)</td>
<td>mg VSS/mg BOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg VSS/mg COD</td>
<td>0.4-0.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Endogenous decay coefficient</td>
<td>b, kd</td>
<td>0.06-0.15</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>g VSS/g VSS.d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Activated Sludge Kinetic Coefficients for Nitrifying Bacteria

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Unit</th>
<th>Value @ 20 °C</th>
<th>Temp. Correction (θ Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum specific growth rate of nitrifying bacteria</strong></td>
<td>$\mu_{mn}$</td>
<td>0.2-0.9</td>
<td>1.06-1.123</td>
</tr>
<tr>
<td><strong>Half-velocity constant for ammonia concentration</strong></td>
<td>$K_n$</td>
<td>0.5-1</td>
<td>1.06-1.123</td>
</tr>
<tr>
<td><strong>Biomass true yield /Synthesis yield coefficient</strong></td>
<td>$Y_n$</td>
<td>0.1-0.15</td>
<td>1.03-1.08</td>
</tr>
<tr>
<td><strong>Endogenous decay coefficient for nitrifying organisms</strong></td>
<td>$k_{dn}$</td>
<td>0.05-0.15</td>
<td>1.04</td>
</tr>
<tr>
<td><strong>Half-velocity constant for dissolved -oxygen concentration</strong></td>
<td>$K_o$</td>
<td>0.40-0.60</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Source: Tale 8-11 Metcalf & Eddy, page 705.