ESM 219

Wastewater Microbiology

Wastewater Microbiology

- Microbes are the catalysts in biological treatment, converting dissolved nutrients to biomass which is then separately disposed of
- Harmful microbes are killed by disinfection prior to releasing the final effluent
- Solids may be digested by anaerobic microbes, producing energy rich gas

Wastewater constituents

Major classes

- Suspended solids (TSS, VSS, etc.)
- Biodegradable organics (BOD, COD
- Pathogens (bacteria, viruses, protozoa, etc.)
- Nutrients (N, P, etc.)
- Priority pollutants (EPA designated toxics)
- Organics that don't biodegrade (refractory)
- Heavy metals (Cd, Zn, Pb, Hg, Cu)
- Ions (pH; contribute to TDS e.g. Ca^{2+}, Na^{+}, SO_4^{-2})
- Temperature
- toxicity

Fig. 7.1. Major contaminants in wastewater. Adapted from Metcalf and Eddy, Inc. (

Biochemical Oxygen Demand (BOD)

- Establishes biological "strength" based on oxygen uptake by microbes during aerobic metabolism of organics in waste
- 5-day (BOD_5) and 20-day (BOD_{20})
- Carbonaceous (CBOD)
- Nitrogenous (NBOD)
- Ultimate (UBOD)

BOD

•Standardized
•Need:
Incubator
Bottles
DO meter
Dilution water
Seed (opt.)
Expressed in mg/L

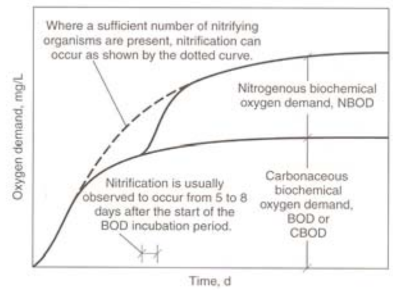
BOD Calculation

$$BOD_5 = [(C_1 - C_2) - ((V_t - V_e)/V_t)(C_3 - C_4)]\ (V_t / V_e)$$

where

- C_1 is the dissolved oxygen concentration, in milligrams per litre, of the test solution at time zero.
- C_2 is the dissolved oxygen concentration, in milligrams per litre, of this same solution after five days.
- C_3 is the dissolved oxygen concentration, in milligrams per litre, of the blank solution at time zero.
- C_4 is the dissolved oxygen concentration, in milligrams per litre, of the blank solution after five days.
- V_e is the volume, in millilitres, of sample used for the preparation of the test solution concerned.
- V_t is the total volume, in millilitres, of this test solution.

Carbonaceous & Nitrogenous BOD



Influent & Effluent BOD & TSS

- Influent (M&E Tbl. 3-13)
  - TSS 100 to 300 mg/L
  - BOD_5 100 to 300 mg/L
- Effluent minimum 2° standards (avg 30-d)
  - TSS 30 mg/L
  - BOD_5 30 mg/L

NOTE: BOD is always less (ca. 0.5) than COD

Nitrogen & Phosphorus

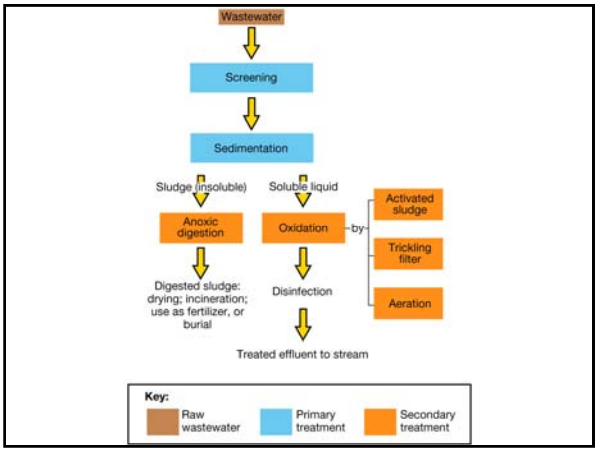
- Nitrogen
  - Urea mineralizes rapidly to NH_4^+
  - NH_4^+ oxidizes to NO_3^- (NBOD)
  - Nitrifying bacteria use CO_2-C (alkalinity)
  - Influent N ranges (mg/L as N)
    - Total: 20 – 70
    - Organic: 8 – 25
    - Ammonia: 12 – 45
    - No nitrate or nitrite typically

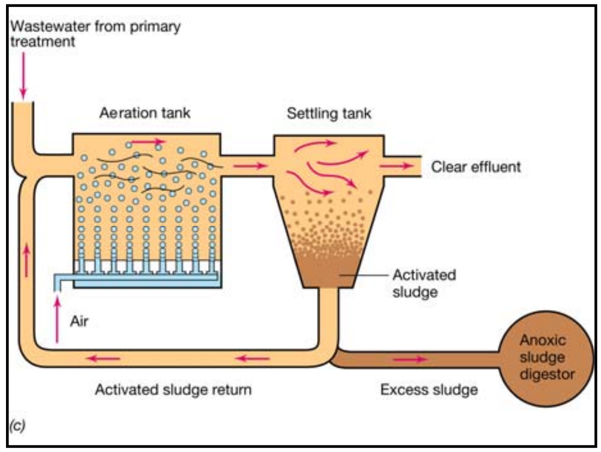
Nitrogen & Phosphorus

- Phosphorus
  - Raw wastewater contains 4 – 16 mg/L as P
  - Effluent may restrict to less than 1 mg/L as P
  - Forms are
    - Orthophosphate: PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4
    - Polyphosphate (polymerized; important *in* biological phosphorous removal)
    - Organic phosphorous (low in raw wastewater)

Biological Constituents

- Many are human pathogens
- Most occupy a role in the treatment process
- They are:
  - Bacteria
  - Archaea
  - Fungi/yeast
  - Protozoa
  - Rotifers
  - Algae
  - viruses





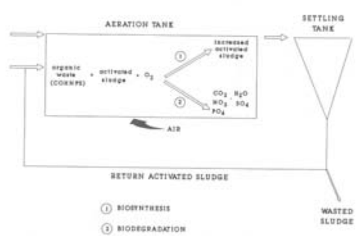
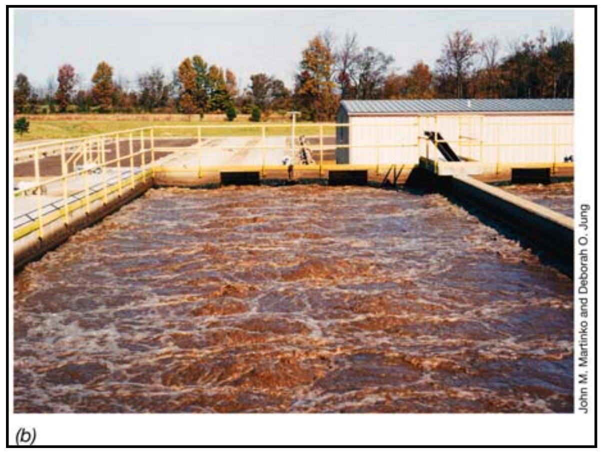


Fig. 8.10. Removal of organic matter by the activated sludge process. Adapted from Curds and Hawkes (1983).



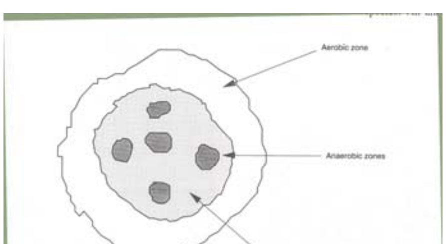


Fig. 8.4. Oxygen distribution in an activated sludge floc. From Wu et al. (1987), with permission of the publisher.

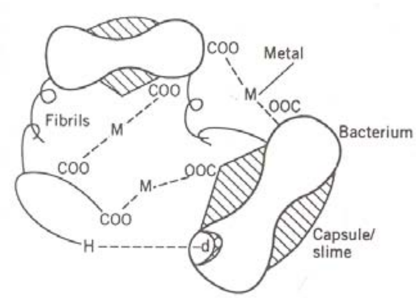


Fig. 8.12. Possible structure of an activated sludge floc. From Forster and Dallas-Newton (1980), with permission of the publisher.

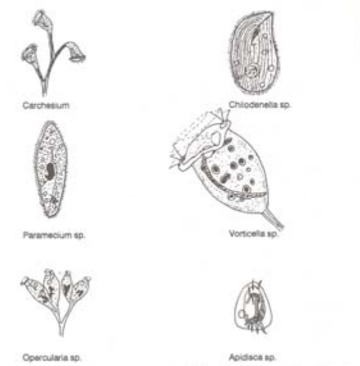


Fig. 8.7. Some protozoa found in wastewater, particularly in activated sludge. Adapted from Dart and Stretton (1980).

Fig. 8.9. Rotifers most commonly found in activated sludge. From Curds and Hawkes (1975), with permission of the publisher.

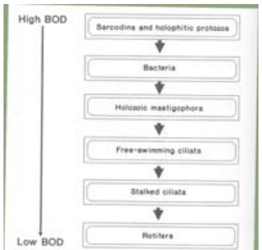


Fig. 8.8. Ecological succession of microorganisms in activated sludge. From Bitton (1980a), with permission of the publisher.

John M. Martinko and Deborah O. Jung

(a)

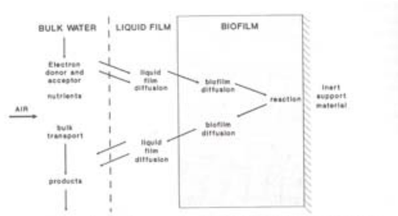


Fig. 10.2. Potentially rate-limiting phenomena involved in biofilm reactions. From Harremoes (1978), with permission of the publisher.

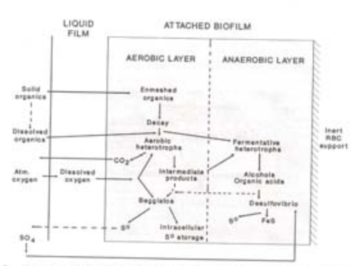


Fig. 10.8. Relationship between *Beggiatoa* and *Desulfovibrio* in an RBC biofilm. Adapted from Alleman et al. (1982).

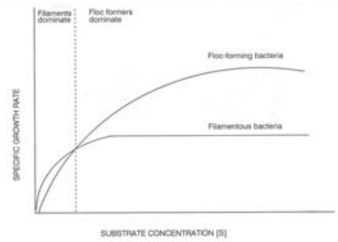
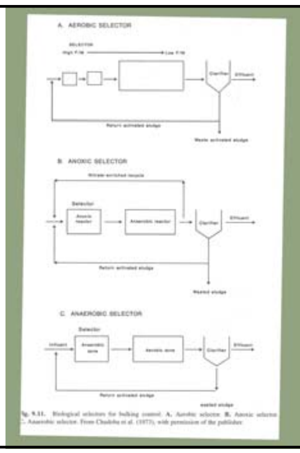


Fig. 9.10. Kinetic selection of filamentous microorganisms based on Monod's equation. From Chudoba et al. (1973), with permission of the publisher.



Biological Nitrification: Stoichiometry

- Ammonia oxidation (nitroso-bacteria)
  $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$
- Nitrite oxidation (nitro-bacteria)
  $2NO_2^- + O_2 \rightarrow 2NO_3^-$
- Overall: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
  = 4.57 g O_2/g N for complete oxidation

Biological Nitrification

- Molecular oxygen required is somewhat less from theoretical N oxidation (synthesis and CO_2 uptake co-occur)
- Nitrifiers need constant concentration of NH_4^+
- Recycle streams (dewatering!) rich in NH_4^+

Nitrification

- Nitrifiers get their C from CO_2 !!!
  - Aerobic chemoautotrophs

  $NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O$
  Theortically 7.14 g alkalinity as $CaCO_3$ used.

Nitrification

- Nitrifiers grow more slowly than aerobic chemoheterotrophs
  - μ_{mn} = 0.25 to 0.77 d^{-1} (20°C); (SRT 4 to 7 days)
  - μ_{m} = 0.6 to 6 d^{-1} (20°C); (SRT 3 to 5 days)
- Nitrifiers are pH sensitive (7.5 to 8.0 = best)
- Nitrifiers are sensitive to toxics
- Nitrifiers are inhibited by ammonia
- Nitrification will occur with long SRTs and warm temperatures.

Biological Nitrogen Removal

- Nitrification generates NO_3^-
- Biological nitrate removal
  - – Assimilatory: nitrate reduced to ammonium and used to make new cells
    - When no NH_4-N is available,
    - Independent of O_2 concentration
  - – Dissimilatory: nitrate respired to ammonia
    - NO_3^- used instead of O_2 as an electron acceptor
    - Occurs in the absence of molecular oxygen

Oxygen: the necessary and expensive ingredient in aerobic biological treatment

- BOD only: $R_o = Q(S_o - S) - 1.42 P_{x,bio}$

Where Ro = oxygen ,kg/d
Px,bio = biomass wasted, kg/d
1.42 is from:

$$\frac{\Delta(O_2)}{\Delta(C_5H_7NO_2)} = \frac{5(32g/mole)}{(113g/mole)} = 1.42\frac{gO_2}{gcells}$$

Oxygen: Rule of thumb

- For BOD removal only, 0.9 to 1.3 kg O_2 per kg BOD is used for SRTs of 5 to 20 days.
- OR 1 lb O_2 per lb BOD_5 applied
- Nitrogen removal greatly increases oxygen demand…….

Denitrification

- Nitrogen conversions

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

- Stoichiometry

$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^-$

$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$

$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$

Important: it puts alkalinity back into the system (half that is lost in nitrification)

Denitrifying Bacteria

- Most are anaerobic chemoheterotrophs
  - – Denitrify in absence of oxygen, but many use oxygen if it is available (facultative)
  - – need organic C
    - For energy
    - For cell synthesis
    - E.g.: sBOD, methanol, acetate
  - – *Pseudomonas* sp. common
- Also autotrophs

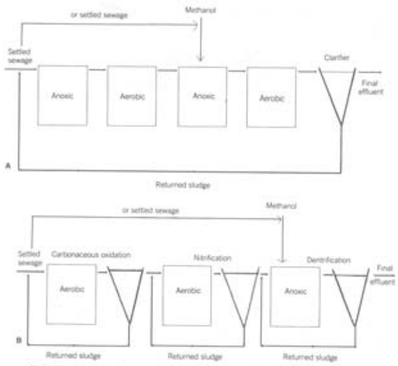


Fig. 8.14. Denitrification systems: A. Single-sludge system B. Multisludge system. From Curds and Hawkes (1983), with permission of the publisher.

Phosphorous removal

- P is limiting nutrient in many aquatic environments; P enrichment leads to eutrophication
- Treatment plant effluent requirements range from 0.1 to 2 mg/L P
- Chemical Treatment (precipitation)
  - Increases operating $
  - Increases sludge production

Biological P Removal

- P and bacteria
  - Macronutrient for bacteria
  - Essential in DNA, ATP, ADP, etc.
  - Cell formula including P: $C_{12}H_{87}O_{23}N_{12}P$
  - 12.2 g N & 2.3 g P per 100 g biomass
  - Many can story poly-P (luxury uptake)
    - Typical content is 2 % of dry weight
    - Poly P results in 20% of dry weight

What leads to excess P storage?

- PAOs recycled as RAS
- Under anaerobic conditions, acetate is formed
- PAOs use acetate and propionate (VFAs) to make PHB (polyester inside the cell), and release orthophosphate
- Under aerobic conditions, PAOs use PHB, make new cells and store poly-P
- Newly recycled cells repeat the cycle.

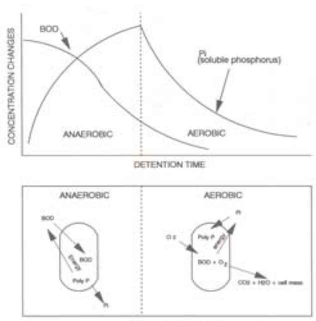
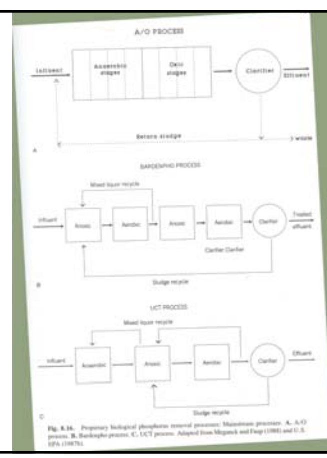
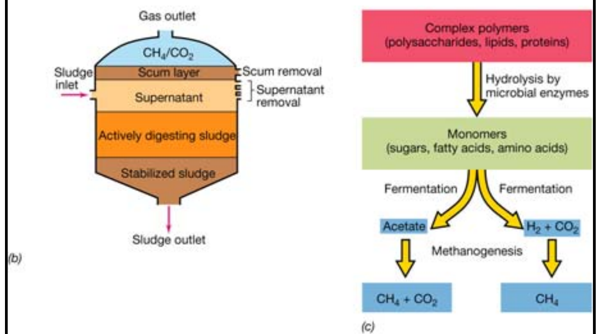


Fig. 8.17. Microbiology of the A/O process. From Deakyne et al. (1984), with permission of the publisher.



(a)

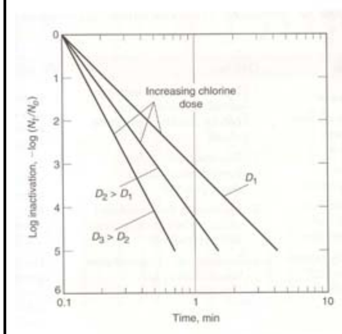
Anaerobic Digestion: Application of methanogenic bacteria for solids stabilization (pathogen reduction)



Disinfection kinetics

$$\frac{dN}{dt} = -k'N \;\; ; \;\; \frac{N}{N_0} = e^{-kt}$$ Chick's Law

$\ln N_t = \ln N_0 - k't$



Time and dose dependency of *E. coli* killing in a batch lab reactor at 20° C.

Chlorine chemistry

- water chemistry is pH dependent
- $Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$ (HOCl at pH<6)
  $HOCl \rightarrow H^+ + OCl^-$ (hypochlorite ion at pH>9)
- Cl- is NOT a disinfectant
- HOCl more powerful, ↓pH most effective (<7.4)
- free available chlorine: HOCl + OCL^-
- calcium hypochlorite [$Ca(OCL)_2$] (70% available chlorine in water); sodium hypochlorite (NaOCl) at 5 to 15% solns

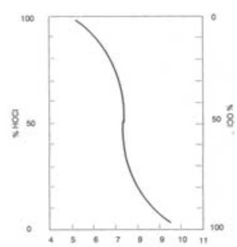


Fig. 6.5. Distribution of HOCl and OCl⁻ in water as a function of pH. From Bitton (1980a), with permission of the publisher.

Chlorine chemistry (cont.)

- chloramines : mono-, di-, tri-: rxn w/ ammonia; kill, but not good disinfectants; combined available chlorine
- $HOCl + NH_3 \rightarrow H_2O + NH_2Cl$ > pH 8.5
- $HOCl + NH_2Cl \rightarrow H_2O + NHCl_2$
- $HOCl + NHCl_2 \rightarrow H2O + NCl_3$ <pH 4.4

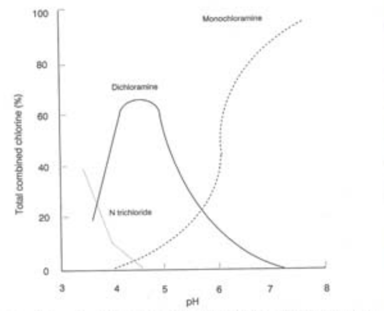


Fig. 6.9. Distribution of types of chloramine with pH. From Wolfe et al. (1984), with permission of the publisher.

Chlorination

- break-point chlorination= addition of enough to achieve free residual
- mechanism: oxidation of SH groups in respiratory enzymes, permeabilty of membranes
- viruses more resistant
- problem is toxic byproducts from residual disinfection by products (DBPs), THM regulation is 80 μg/L

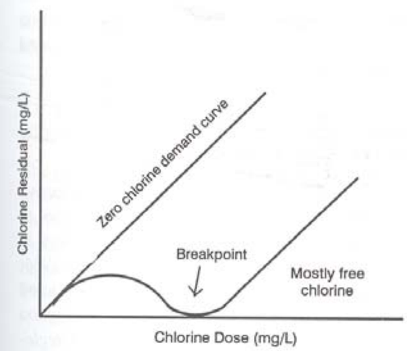


Fig. 6.10. Dose–demand curve for chlorine-ammonia reaction after 1 hr at pH 7–8. From Kreft et al. (1985), with permission of the publisher.

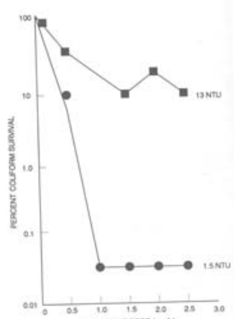


Fig. 6.3. Effect of turbidity on coliform persistence in chlorinated water. Adapted from LeChevallier et al. (1981).

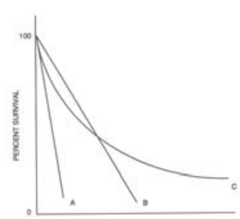


Fig. 6.1. Inactivation curves of microorganisms following disinfection. A. Sensitive homogeneous population. B. More resistant homogeneous population. C. Heterogeneous population or one partially protected by aggregation. From Hoff and Akin (1986), with permission of the publisher.

Chlorine Dioxide: more virucidal

- generated by reacting chlorine and sodium chlorite:
  - $Cl_2 + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl$
- pH 6-8: $2ClO_2 + 2OH_2 \rightarrow ClO_2^- + ClO_3^-$

  chorite chlorate
- effective as HOCl and more than OCl^- or chloramines
- disrupts protein synthesis
- doesn't form toxic chloramines, or THMs
- EPA regulates residual chlorite and chlorate to 1 mg/L in drinking H_2O

Dechlorination w/ sulfur dioxide

- *Rxns with chlorine:*
  - $SO_2 + H_2O \rightarrow HSO_3^- + H^+$
  - $HOCl + HSO_3^- \rightarrow Cl^- + SO_4^{-2} + 2H^+$
  - $SO_2 + HOCl + H_2O \rightarrow Cl^- + SO_4^{-2} + 3H^+$
- *Rxns with chloramines:*
  - $SO_2 + H_2O \rightarrow HSO_3^- + H^+$
  - $NH_2Cl + HSO_3^- + H_2O \rightarrow Cl^- + SO_4^{-2} + NH_4^+ + H^+$
  - $SO_2 + NH_2Cl + 2H_2O \rightarrow Cl^- + SO_4^{-2} + NH_4^+ + 2H^+$
- *Rxns with chlorine dioxide*
  - $SO_2 + H_2O \rightarrow H_2SO_3$
  - $H_2SO_3 + 2ClO_2 + H_2O \rightarrow 5H_2SO_4 + 2HCl$

Ozone Disinfection

$O_3 + H_2O \rightarrow HO_3^+ + OH^-$

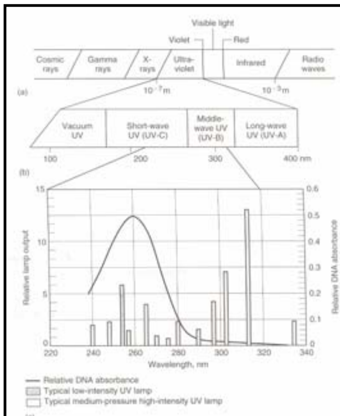
$HO_3^+ + OH^- \rightarrow 2HO_2$

$O_3 + HO_2 \rightarrow HO + 2O_2$

$HO + HO_2 \rightarrow H_2O + O_2$

UV Disinfection

- optimum germicidal λ = 250- 270 nm
- Typically Hg vapor (output is 253.7 nm).
- Physical method: radiation absorbed by DNA and RNA, death and inability to replicate
- First order process.
- Time and distance dependent.
- Nature of liquid, competing reactions & turbidity affect.



UV Radiation:

220 to 320 nm = germicidal

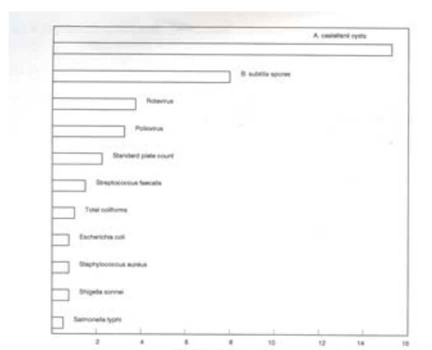
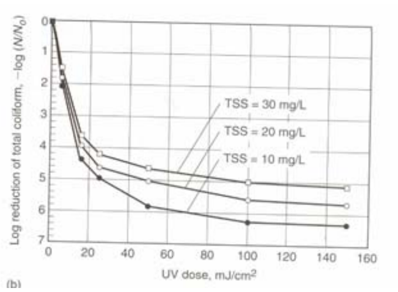


Fig. 6.16. Relative UV dose required to inactive 99.9% of various microorganisms compared to that for *E. coli*. From Chang et al. (1985), with permission of the publisher.

Turbidity (TSS) Dependency



Effect of Organic Wastes on Stream Ecosystems

- **Streeter-Phelps Model – DO sag curve**
  - Many equations and computer programs are available today to describe the quality of water in streams, rivers and lakes
  - The most prevalent is the Streeter Phelps equation.
  - Addition of wastewater (BOD) typically causes a slow decrease in O_2, followed by a gradual increase close to the dissolved oxygen (D.O.) saturation concentration

Streeter-Phelps Model

- Assumptions of the Model
  - stream is an ideal plug flow reactor
  - steady-state flow and BOD and DO reaction conditions
  - The only reactions of interest are BOD exertion and transfer of oxygen from air to water across air-water interface

Streeter-Phelps Model

- Mass Balance for the Model
  - Not a Steady-state situation

rate O_2 accum. = rate O_2 in – rate O_2 out + prod. – cons.

rate O_2 accum. = rate O_2 in – 0 + 0 – rate O_2 cons.

- Both reoxygenation and deoxygenation are 1st order

rate of deoxygenation = $-k_1C$

k_1= deoxygenation constant, function of waste type and temperature

Streeter-Phelps Model

rate of reoxygenation = k_2D

D = deficit in D.O. or difference between saturation and current D.O.

k_2 = reoxygenation constant

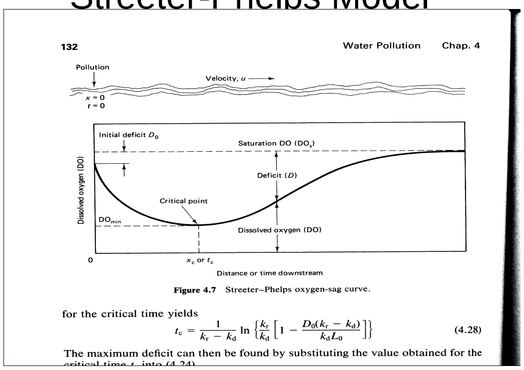
$$k_2 = \frac{3.9 v^{1/2}\left([1.025]^{(T-20)}\right)^{1/2}}{H^{3/2}}$$

Streeter-Phelps Model

- Where
  - T = temperature of water, ºC
  - H= average depth of flow, m
  - v = mean stream velocity, m/s
- Oxygen Deficit
  - D = S – C

D.O. deficit = saturation D.O. – D.O. in the water

Streeter-Phelps Model



Streeter-Phelps Model

- Deoxygenation rate is equivalent to BOD of waste
  - $r_o = k_1 L_t$
  - $L_t = L_o e^{-Kt}$

L_o or L = ultimate BOD of the wastewater and stream water mixture

Streeter-Phelps Model

- In terms of the deficit with time

$$\frac{dD}{dt} = k_1 z - k_2 D$$

$$z = L e^{-k_1 t}$$

Streeter-Phelps Model

- Substituting and integrating yields the following equations

$$D = \frac{k_1 L_o}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + D_o e^{-k_2 t}$$

$$\frac{dD}{dt} = k_1 L_o e^{-k_1 t} - k_2 D = 0$$

$$D_c = \frac{k_1}{k_2} L_o e^{-k_1 t}$$

$$t_c = \frac{1}{k_2 - k_1} \ln \left\{ \frac{k_2}{k_1} \left[1 - \frac{D_o (k_2 - k_1)}{k_1 L_o} \right] \right\}$$

Streeter-Phelps Model

- Example:
  - Wastewater mixes with a river resulting in a

BOD = 10.9 mg/L, DO = 7.6 mg/L

The mixture has a temp. = 20 °C

Deoxygenation const.= 0.2 day^{-1}

Average flow = 0.3 m/s, Average depth = 3.0 m

DO saturated = 9.1 mg/L

- Find the time and distance downstream at which the oxygen deficit is a maximum?
- Find the minimum value of DO?

Streeter-Phelps Model

- Initial Deficit

D_o = 9.1 – 7.6 = 1.5 mg/L

- Estimate the reaeration constant

$$k_2 = \frac{3.9 v^{1/2}}{H^{3/2}} = \frac{3.9(0.3 m/s)^{1/2}\left([1.025]^{(20-20)}\right)^{1/2}}{(3.0 m)^{3/2}} = 0.41 day^{-1}$$

Streeter-Phelps Model

- Calculate the time at which the maximum deficit is reached, with t_c:

$$t_c = \frac{1}{k_2 - k_1} \ln\left\{\frac{k_2}{k_1}\left[1 - \frac{DO_o(k_2 - k_1)}{k_1 L_o}\right]\right\}$$

$$= \frac{1}{(0.41 - 0.2)} \ln\left\{\frac{0.41}{0.2}\left[1 - \frac{1.5(0.41 - 0.2)}{0.2 \times 10.9}\right]\right\}$$

$$= 2.67 \text{days}$$

$$x_c = vt_c = 0.3 \text{m/s} \times 86{,}400 \text{s/day} \times 2.67 \text{days} = 69{,}300 \text{m}$$

Streeter-Phelps Model

- The maximum DO deficit is:

$$D_c = \frac{k_1}{k_2} L_o e^{-k_1 t}$$

$$= \frac{0.2}{0.41} (10.9 \text{ mg/L}) e^{-(0.2 \text{day}^{-1})(2.67 \text{days})}$$

$$= 3.1 \text{mg/L}$$