Lecture 16, 17 – Biological Reaction Kinetics

From: P.L. McCarty 1975 Stoichiometry of Biological Reactions
Progress in Water Technology, Vol 7, No 1, Pp. 157-172

Chemical equation for biological oxidation of wastes:

$$\underset{\substack{\text{Casein} \\ \text{(in dairy waste)}}}{C_8H_{12}N_2O_3} + 3O_2 \xrightarrow{\text{bacteria}} \underset{\substack{\text{New} \\ \text{cells}}}{C_5H_7O_2N} + NH_3 + 3CO_2 + H_2O \qquad (1)$$

Alternative representation of cells is $C_{60}H_{87}O_{23}N_{12}P$

Note, Redfield ratio is $C_{106}N_{16}P$

Algae = $C_{106}H_{263}O_{110}N_{16}P$

Above reaction requires bacteria to catalyse the reaction

Type of bacteria in this reaction are aerobic heterotrophs

Heterotrophic microbes use organic carbon as energy and carbon source for new growth

Autotrophic microbes use CO_2 as carbon source (e.g. algae)

Aerobic microbes use oxygen as an electron acceptor

Anaerobic microbes use something other than oxygen as electron acceptor

Anoxic microbes use nitrate or nitrite reduction to N_2 (denitrification)

Microbes may be obligate aerobes – able to use O_2 only – or facultative – able to use O_2 or NO_2^-/NO_3^-

To understand electron acceptor concept, it is helpful to break down Equation 1 into synthesis and energy components

Synthesis (of new cells)

$$\frac{1}{2} C_8H_{12}N_2O_3 + \frac{1}{8} H_2O \rightarrow C_5H_7O_2N + \frac{1}{4} NH_3$$

Energy generation

$$\frac{3}{8} C_8H_{12}N_2O_3 + 3O_2 \rightarrow 3CO_2 + \frac{3}{4} NH_3 + \frac{9}{8} H_2O$$

Disassemble into half reactions to highlight electron donors and acceptors

Synthesis

Donor $\frac{5}{8} C_8H_{12}N_2O_3 + \frac{65}{8} H_2O \rightarrow 5CO_2 + \frac{10}{8} NH_3 + 20H^+ + 20e^-$

Acceptor $5CO_2 + NH_3 + 20H^+ + 20e^- \rightarrow C_5H_7O_2N + 8H_2O$

Energy

Donor $\frac{3}{8} C_8H_{12}N_2O_3 + \frac{39}{8} H_2O \rightarrow 3CO_2 + \frac{3}{4} NH_3 + 12H^+ + 12e^-$

Acceptor $3O_2 + 12H^+ + 12e^- \rightarrow 6H_2O$

These equations can be normalized such that there is a single electron on right-hand side of each equation

Cells $\frac{1}{20} C_5H_7O_2N + \frac{2}{5} H_2O = \frac{1}{4} CO_2 + \frac{1}{20} NH_3 + H^+ + e^-$

Donor $\frac{1}{32} C_8H_{12}N_2O_3 + \frac{13}{32} H_2O = \frac{1}{4} CO_2 + \frac{1}{16} NH_3 + H^+ + e^-$

Acceptor $\frac{1}{2} H_2O = \frac{1}{4} O_2 + H^+ + e^-$

This reorganization of the equations illustrates:

Energy is required to create new cells

Energy is created in the electron donor to electron acceptor transfer

$C_8H_{12}N_2O_3$ acts as an electron donor (there are many others as well)

O_2 acts as an electron acceptor
Other anaerobic electron acceptors are:

NO_3 (denitrification)
Fe
SO_4 ↓ decreasing energy

Differences in energy production associated with different electron acceptors is illustrated by reactions of glucose on page 4. Aerobic oxidation is most favorable, denitrification close, and others very inferior in terms of free energy produced

Source for slide: Bruce E. Rittman and Perry L. McCarty, 2001 Environmental Biotechnology: Principals and Applications. McGraw-Hill, New York.

Reactions shown above are for casein ($C_8H_{12}N_2O_3$) and glucose ($C_6H_{12}O_6$)

Generic representation of municipal wastewater is: $C_{10}H_{19}O_3N$

No actual compound corresponds to this formula hence no evaluation of energy, etc. is possible

FREE ENERGY
kJ/mol GLUCOSE

Aerobic Oxidation

$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$ -2,880

Denitrification

$5\ C_6H_{12}O_6 + 24\ NO_3^- + 24\ H^+ \longrightarrow 30\ CO_2 + 42\ H_2O + 12\ N_2$ -2,720

Sulfate Reduction

$2\ C_6H_{12}O_6 + 6\ SO_4^{2-} + 9\ H^+ \longrightarrow 12\ CO_2 + 12\ H_2O + 3\ H_2S + 3\ HS^-$ -492

Methanogenesis

$C_6H_{12}O_6 \longrightarrow 3\ CO_2 + 3\ CH_4$ -428

Ethanol Fermentation

$C_6H_{12}O_6 \longrightarrow 2\ CO_2 + 2\ CH_3CH_2OH$ -244

Figure by MIT OCW.

Adapted from: Rittman, Bruce E., and Perry L. McCarty. *Environmental Biotechnology: Principals and Applications*. New York, NY: McGraw-Hill, 2001.

Part of biological oxidation goes to bacterial growth

Bacterial growth requires:

1. Carbon source to form cellular material
2. Energy source to fuel cell synthesis
   - Phototrophs get energy from light
   - Chemotrophs get energy from chemical reactions
     - Chemoautotrophs from inorganic chemical reactions
       (e.g. nitrifying bacteria use ammonia and nitrite:
       $$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$
       $$2NO_2^- + O_2 \rightarrow 2NO_3^-$$
     - Chemoheterotrophs from oxidation of organics
   - If chemotrophs use an external electron acceptor they have a respiratory mechanism ($e.g.\ O_2,\ NO_3^-,\ Fe^{2+},\ SO_4^-$)
   - If chemotrophs use an internal (organic) electron acceptor they have a fermentive mechanisms
3. Nutrient source to form cell material
   - Macronutrients – N and P
   - Other major nutrients – S K Mg Ca Fe Na Cl
   - Minor nutrients – Zn Mn Mo Se Cu Ni

Bacteria grow rapidly –

Bacteria reproduce in <20 min to several days (generation time)

One bacterium with 30 min generation time

$\rightarrow 24^2 = 16.8$ million in 12 hours

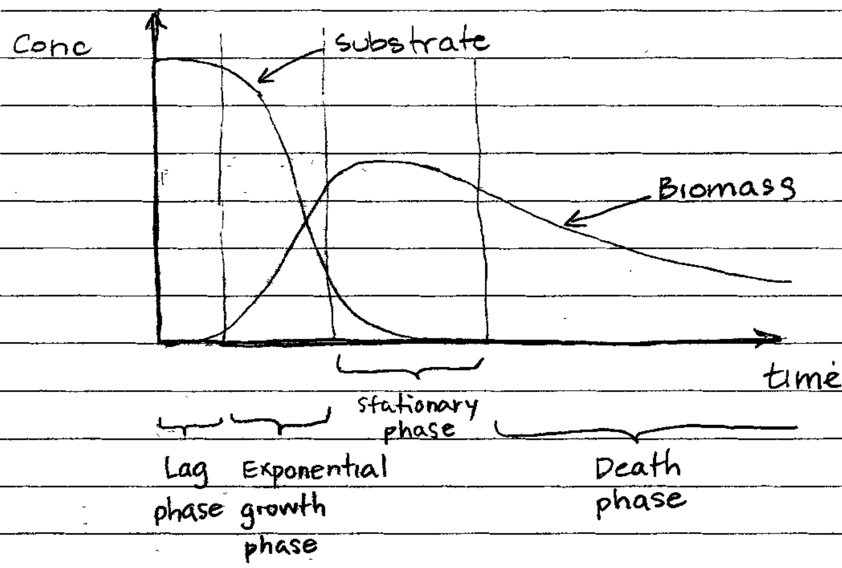
Mass: 5×10^{-13} g $\rightarrow$ 8×10^6 g $= 8\ \mu g$

Large numbers not necessarily mass

Mass calculation assumes $1\ \mu m$ sphere with $\rho = 1\ g/cm^3$

Bacteria growing at high rates sooner or later outgrow available resources

In batch cultures (fixed quantity of biodegradable organics and nutrients with no inflow) growth looks like:



Biological wastewater treatment depends on balance between substrate and biomass – ideally, biological reactor will operate in stationary growth phase

Need to understand:

1. How much substrate yields how much biomass

2. How quickly substrate is used

1. Biomass yield

$$Y = \frac{\text{mass biomass produced}}{\text{mass substrate consumed}}$$

A. Can determine yield from measurements

Organic matter in waste is measured as BOD or COD (discussed further below)

Biomass is taken to be VSS - volatile suspended solids

TSS = total suspended solids
= mass of solids captured on 1.58 μm glass-fiber filter

VSS = volatile suspended solids
= mass of solids burned off at 500°C

FSS = fixed suspended solids
= residual after ignition
= TSS - VSS

TS = total solids
= mass of residue after evaporation and drying at 104°C

TDS = total dissolved solids
= mass of solids that pass through filter and remain after drying at 104°C

TS = TSS + TDS

B. Can determine yield from stoichiometry

E.g. glucose → cells

$$3\,C_6H_{12}O_6 + 8O_2 + 2NH_3 \rightarrow 2C_5H_7NO_2 + 8CO_2 + 14H_2O$$

MW:

$$3(180) \quad 8(32) \quad 2(17) \qquad 2(113)$$

Yield in terms of glucose:

$$Y = \frac{2 \text{ moles } (113 \text{ g/mol})}{3 \text{ moles } (180 \text{ g/mol})} = 0.42 \ \frac{\text{g cells}}{\text{g glucose}}$$

Yield in terms of COD:

COD is Chemical Oxygen Demand = amount of oxygen needed to fully oxidize the substrate

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

$$\text{COD: } \frac{\Delta O_2}{\Delta C_6H_{12}O_6} = \frac{6 \text{ mol} \cdot 32 \text{ g/mol}}{1 \text{ mol} \cdot 180 \text{ g/mol}}$$

$$= 1.07 \ \frac{\text{g COD}}{\text{g glucose}}$$

Yield for COD

$$Y = \frac{2 \text{ moles} \cdot 113 \text{ g/mol}}{3 \text{ mol glucose} \cdot 180 \text{ g/mol glucose} \cdot 1.07 \text{ g } O_2/\text{g glucose}}$$

$$= 0.39 \ \frac{\text{g cells}}{\text{g COD}}$$

Actual yields are less since cells use some substrate for energy to maintain cell

c. Can determine yield from bioenergetics

Compute Gibbs free energy for synthesis (cell production) and energy generation components of reaction

This yields equation for mole of substrate generated per mole of substrate used.

See Metcalf & Eddy for details

Method 1 is best, but requires field, pilot or lab installation
Methods 2 and 3 provide theoretical context, predictive ability

For design, also need to know O_2 requirement

O_2 is used to convert glucose to energy and to create biomass

From stoichiometry

$$\underset{3(180)}{3C_6H_{12}O_6} + \underset{8(32)}{8O_2} + 2NH_3 \rightarrow 2C_5H_7NO_2 + 8CO_2 + 14H_2O$$

$$\frac{O_2 \text{ used}}{\text{glucose}} = \frac{8 \text{ mol} \cdot 32 \text{ g } O_2/\text{mol}}{3 \text{ mol} \cdot 180 \text{ g glucose}/\text{mol}}$$

$$= 0.474 \frac{\text{g } O_2}{\text{g glucose}}$$

$$\frac{O_2 \text{ used}}{\text{COD}} = 0.474 \frac{\text{g } O_2}{\text{g gluc.}} \Big/ 1.07 \frac{\text{g COD}}{\text{g gluc.}}$$

$$= 0.44 \frac{\text{g } O_2}{\text{g COD used}}$$

Why is this not $1.0 \frac{\text{g } O_2}{\text{g COD}}$?

Difference is in COD represented by cells

COD of cells is:

$$\underset{113}{C_5H_7NO_2} + \underset{5(32)}{5O_2} \rightarrow 5CO_2 + NH_3 + 2H_2O$$

$$\frac{\text{g COD}}{\text{g cell}} = \frac{5(32)}{113} = 1.42 \frac{\text{g COD}}{\text{g cell}}$$

Cell yield showed $Y = 0.42 \dfrac{\text{g cells}}{\text{g gluc}}$

$$= \frac{0.42 \dfrac{\text{g cells}}{\text{g gluc}} \times 1.42 \dfrac{\text{g COD}}{\text{g cells}}}{1.07 \dfrac{\text{g COD}}{\text{g gluc}}}$$

$$= 0.56 \frac{\text{g COD cells}}{\text{g COD gluc}}$$

Of 1 g COD entering as glucose, 0.56 goes into producing COD as cells and 0.44 is oxidized by O_2

Waste is often expressed as BOD - biochemical oxygen demand

BOD captures three processes:

Oxidation to produce energy:

$$\text{Waste} + O_2 \xrightarrow{\text{bact.}} CO_2 + H_2O + NH_3 + \text{other end products} + \text{energy}$$

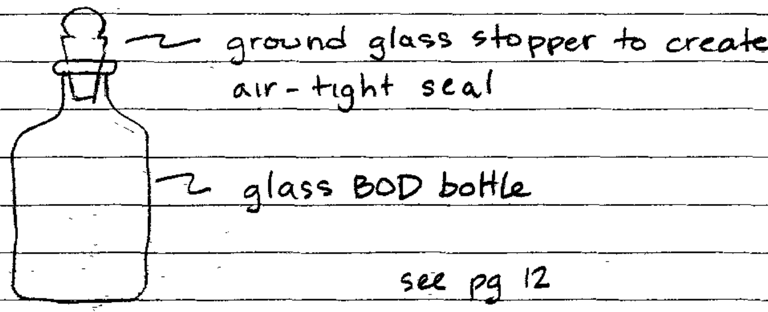
Cell synthesis:

$$\text{Waste} + O_2 + \text{energy} \xrightarrow{\text{bact.}} C_5H_7NO_2$$

Endogenous respiration (cell's use of own biomass to get energy for cell maintenance)

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

BOD is measured in a standard bottle test:



see pg 12

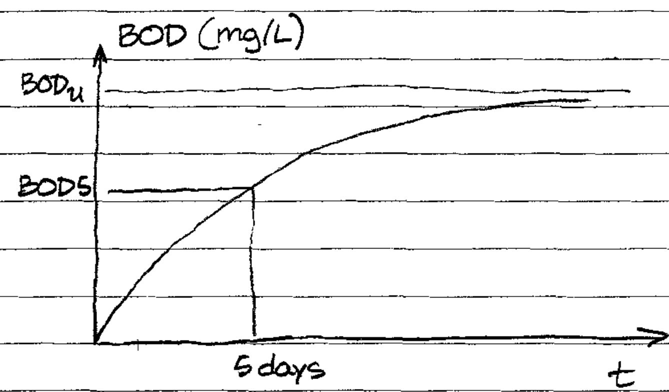
Wastewater + bacteria "seed" put in bottle
Dissolved oxygen (DO) concentration measured
Bottle is sealed and incubated for t days
DO is measured at end of t days
$\Delta DO = BOD$

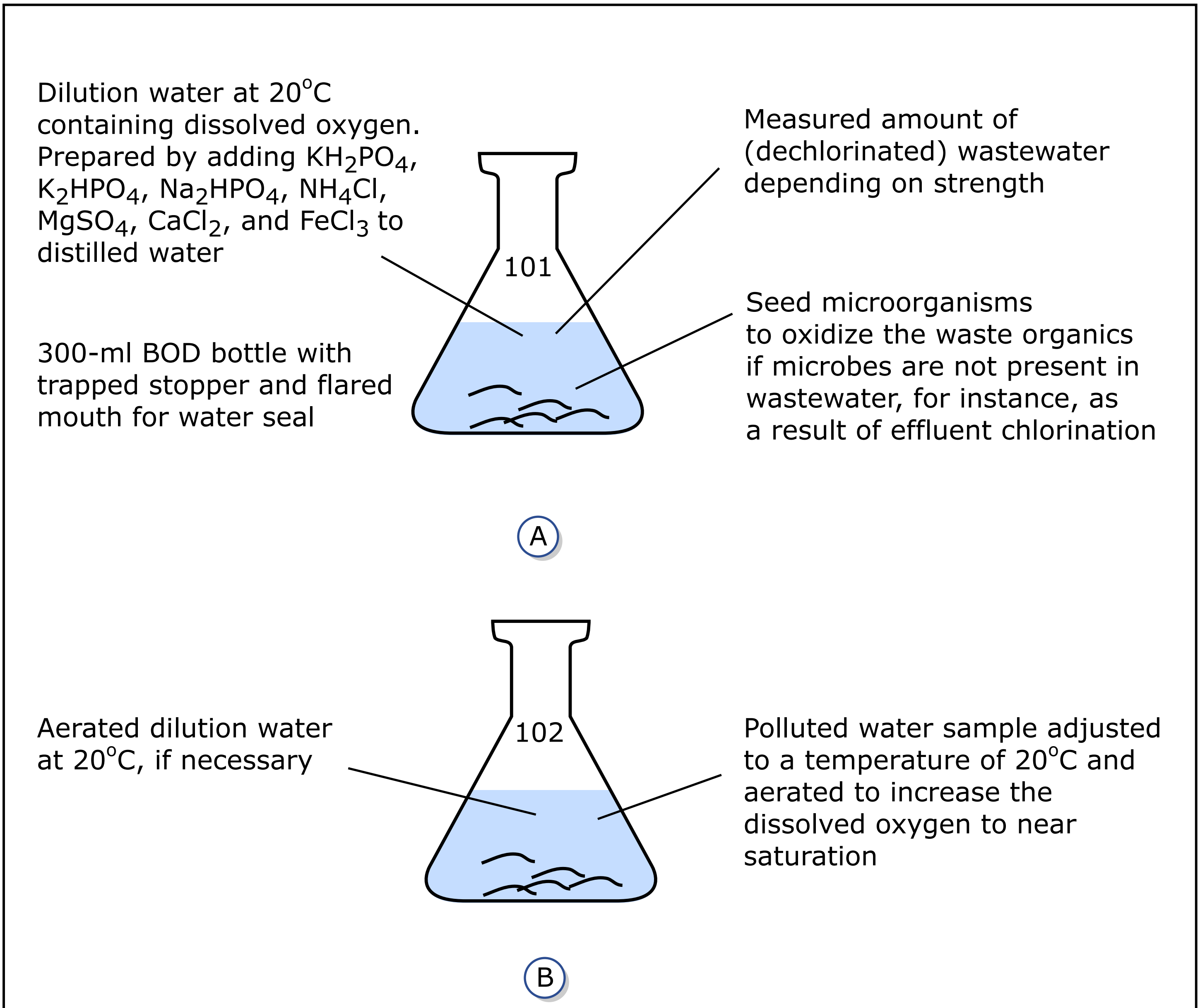
t is traditionally 5 days = BOD5

t is sometimes 20 days = BOD20
used when BOD5 is too low to measure or for slowly degrading waste

t is occasionally very long – 100+ days
used for papermill wastewater, other wastewaters with very slowly degrading wastes – known as long-term BOD tests = BODu "ultimate BOD"
or UBOD

BOD develops over time:





Preparation of biochemical oxygen demand (BOD) tests on (A) Wastewater sample, (B) Polluted surface water.

Figure by MIT OCW.

Adpated from: Viessman, W., Jr., and M. J. Hammer. *Water Supply and Pollution Control*. 7th ed. Upper Saddle River, NJ: Pearson Education, Inc., 2005, p. 318.

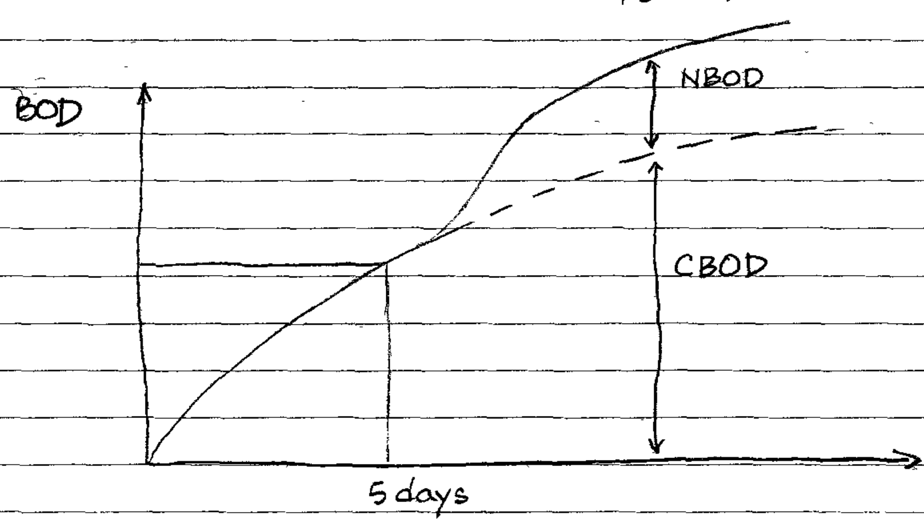
BOD curve vs. t follows first-order relation

$$BOD_t = BOD_u \left(1 - e^{-k_1 t}\right)$$

k_1 = deoxygenation coefficient
(note V & H defines this in terms of base 10, but base e is more conventional)

As BOD is consumed (biodegraded) in bottle, DO is also consumed. For long tests, bottle needs to be reaerated (measuring DO before and after) occasionally to prevent creation of anaerobic conditions

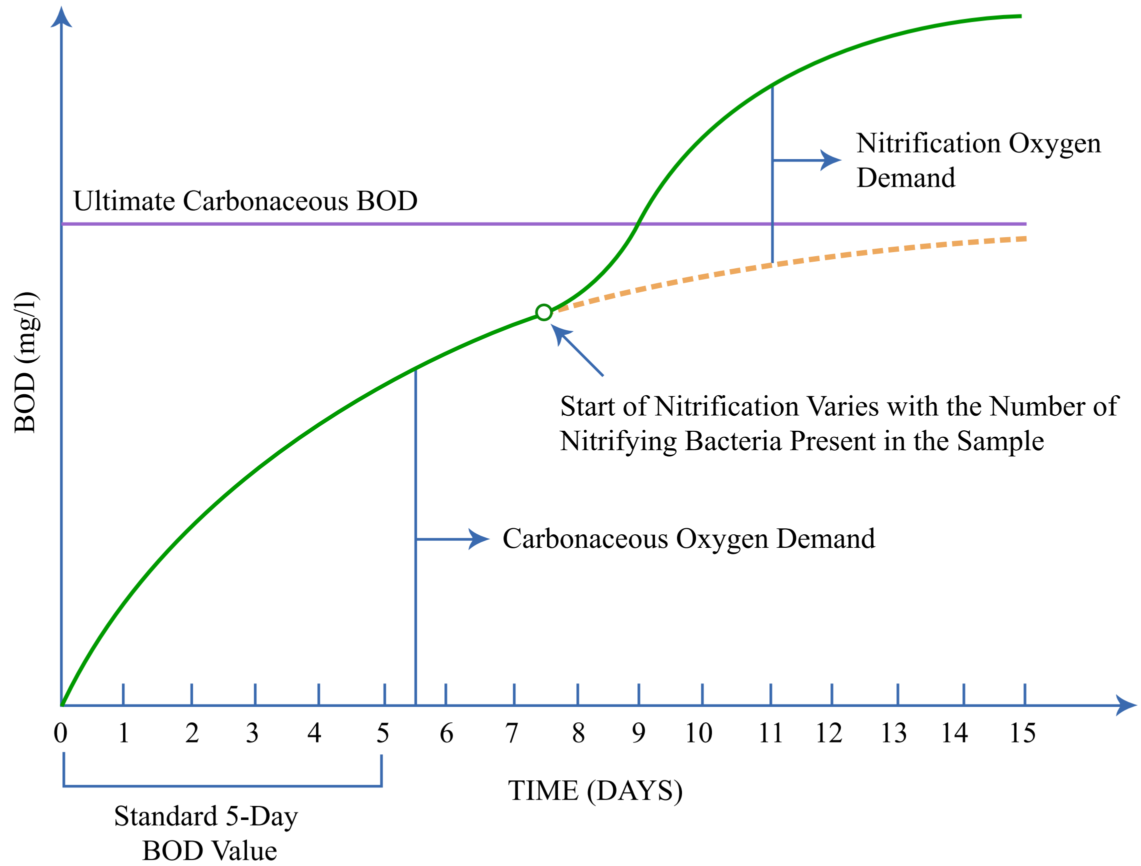
Actual BOD test is not as simple as shown. Real BOD tests look like: (see pg 14)



NBOD represents oxygen demand by nitrifying bacteria converting ammonia to nitrate:

$$2\,NH_4^+ + 3O_2 \xrightarrow{\text{nitrosomonas}} 2NO_2^- + 4H^+ + 2H_2O$$

$$2NO_2^- + O_2 \xrightarrow{\text{nitrobacter}} 2NO_3^-$$



Hypothetical biochemical oxygen demand reaction curve showing the cabornaceous & nitrification reactions.

Figure by MIT OCW.

Adpated from: Viessman, W., Jr., and M. J. Hammer. *Water Supply and Pollution Control*. 7th ed. Upper Saddle River, NJ: Pearson Education, Inc., 2005, p. 319.

NBOD can be determined stoichiometrically from net nitrification reaction:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O$$

1 gm NH_4 consumes 4.57 gm oxygen

NBOD can be suppressed by nitrification inhibitors added to BOD bottle at start of test

How do BOD and COD relate?

COD is measured by chemical test – dichromate $Cr_2O_7^{2-}$ (a strong oxidant) is added, reacted with organics, and leftover dichromate measured by titration
By subtraction, dichromate used to oxidize is computed and converted to equivalent O_2

COD and BOD are fundamentally different:

COD is defined chemical quantity
BOD is a bioassay
Not necessarily correlated

For untreated municipal wastewater

$$\frac{BOD}{COD} \simeq \frac{2}{3}$$ is often assumed

For more information see: Rodger B. Baird and Roy-Keith Smith, 2002. Third Century of Biochemical Oxygen Demand. Water Environment Federation, Alexandria, Virginia.

Typical BOD values	COD (mg/L)	CBOD5 (mg/L)	NBOD (mg/L)	BOD5/COD
Municipal wastewater				
untreated	450	200	220	0.3 - 0.8
primary treatment	250	130		0.4 - 0.6
secondary treatment	50	30	40	0.1 - 0.3
Combined sewer overflow	370	170	290	

Source: US EPA, 1997 Technical Guidance Manual for Developing Total Maximum Daily Loads, Book 2: Streams and Rivers, Part 1: Biochemical Oxygen Demand/Dissolved Oxygen and Nutrients/Eutrophication. Report No. EPA-823-B-97-002.