This unit provides an introduction to basic concepts which are applicable to all biological treatment systems. It consists of three lessons which focus on: the microorganisms found in biological systems; the factors that affect the growth and health of biological systems; and the interrelationship between groups of microorganisms, their competition, predominance, and symbiosis. The instructor's guide for the unit includes: (1) an overview of the unit; (2) lesson plans; (3) lecture outlines (keyed to a set of slides designed for use with the lessons); (4) a student worksheet for each lesson (with answers); and (5) two copies of a final quiz (with and without answers). (JN)
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CONCEPTS OF BIOLOGICAL TREATMENT

Overview

This section is intended to be used introductory to the more specific biological treatment processes that follow. Material in this section is basic and applies to all biological treatment systems. This section could also be used in conjunction with single lessons. The section is presented in three lessons: #1 The Microorganisms; #2 Environmental Factors; and #3 Population Dynamics. Lesson 1 is a survey of the microorganisms found in biological systems. Lesson 2 deals with the factors that affect the growth and health of biological systems. Lesson 3 covers the interrelationship between groups of microorganisms, their competition, predominance and symbiosis.

Lesson Plans

Lesson 1 - The Microorganisms
- Have students read text material ahead of time, if possible
- Lecture from outline with slide support (about 45 min)
- Assign worksheet (10 min)
- Correct and discuss worksheet (15 min)

Lesson 2 - Environmental Factors
- Have students read text material ahead of time, if possible
- Lecture from outline with slide support (about 30 min)
- Assign worksheet (10 min)
- Correct and discuss worksheet (10 min)

Lesson 3 - Population Dynamics
- Have students read text material ahead of time, if possible
- Lecture from outline with slide support (about 30 min)
- Assign worksheet (10 min)
- Correct and discuss worksheet (10 min)
- Assign final quiz (15 min)
Lesson 1 - The Microorganisms

Slide #

#1 & #2 Title & Credit Slides

#3 The Biological System
- The flow
- The sound
- The "dirty, foaming water"
- The "Bugs"

#4, #5, #6 To "See" the "Bugs" - Consider the Objectives of Wastewater Treatment

#7 Protect receiving stream
- Prevent oxygen depletion

#8 Reduce food material in waste
- The "Bugs" use food in waste

#9 Oxygen supplied

#10 "Bugs" eat and eat
- Clean water discharged

#11, #12 Drawback - "Bugs" grow - too many
- Excess must be separated and disposed of

The Operator's main job

#13, #14 Keep "bugs" comfortable and happy
- Must have enough to eat
- Correct environment
- Free of disease
- Free of poison
- Free of predators
What are these "bugs"?

A mixed biological system

Workers
Troublemakers
Freeloaders

You can find -

Bacteria
Protozoa
Viruses
Fungi
Algae
Other higher forms

Bacteria

Most numerous
Most important to treatment
Single-cells
1 micron (micrometer; $10^{-6}$ m) long

Shapes

cocci - spheres
bacilli - rods
spirals

Motility

gliding
flagella

Capsules

Protozoa

Microscopic animals
Single-cells
Much larger than bacteria
More complex than bacteria

4 types

Amoeba (Sar<odina)
no definite shape
false feet
amoeboid movement
Ciliates (Ciliata)
Free-swimming
cilia
ovoid to pear-shaped
Stalked
cup-shaped cell with a stalk
cilia around "cup"

Flagellates (Mastigophora)
Flagella
Free-swimming

Suctoreans (Suctoria)
Rigid tentacles
Stalked

Viruses
Strictly parasitic
Lack metabolic systems
Extremely small
Genetic material and a protective coat

Fungi
Similar to bacteria

Algae
Microscopic plants
Photosynthetic

Higher forms
Rotifers
Snails
Worms
Insect larvae and adults
Crustaceans

Review Slides

I-CB-5
Lesson 2 - Environmental Factors

Slide #

#1 & #2 Title and Credit Slides

Review of Biological Process

#3 What does the operator really see?
Population of mixed microorganisms

#4 Organisms consume organics, use oxygen and remove dissolved and suspended solids before they get to the receiving stream

#5 The health of the population depends on several factors:
- Oxygen
- Nutrients
- Flow
- Temperature
- pH
- Toxics

#6 Outline Slide - Food

#7 The food (nutrients) comes from many sources in the collection system

#8 The nutrients (organic food) directly affect the amount of growth; number of microorganisms

- More cells with more food

#9 Typical batch growth curve

- Increase in microorganisms vs time after a single dose of food

#10 Log growth phase - exponential growth unlimited by food supply

#11 Declining growth phase - food starts to limit the rate of cell increase
Endogenous growth phase - food is limiting; bacteria use stored nutrients to survive.

Food to microorganism ratio - F/M ratio - high at first then become small.

Other nutrient requirements
- How easily are the organics used?
- Inorganic minerals and trace elements

Outline Slide - Hydraulic Load
- Affects solids and nutrient concentration
  - Low flow - high concentration
  - High flow - low concentration

Outline Slide - Oxygen
- Aerobic conditions - use free, dissolved oxygen
- Anaerobic conditions - use chemically bound oxygen
- Facultative - aerobic or anaerobic conditions - bacteria can use either form of oxygen
  - Under aerobic conditions aerobic and facultative organisms present
  - Under anaerobic conditions, anaerobic and facultative organisms present
  - If oxygen is present the facultative organisms will use aerobic respiration and convert organics at a faster rate.
Outline Slide - Temperature

Three temperature growth ranges
thermophilic
mesophilic
psychrophilic

Temperature vs growth rate
Organisms have an optimum growth temperature

Efficiency increases as temperature increases
Growth rate doubles for each 10 degrees increase

Outline Slide - pH
Most organisms prefer pH near neutral
Fungi grow well at lower pH

pH vs growth rate
Organisms have an optimum growth pH

Outline Slide - Toxics
Toxics can inhibit growth or completely stop growth

Review Slide of growth factors
All of these factors affect the bio-system

The operator must control these growth factors in order to keep the bio-system going down the path of optimum treatment

It is only with a knowledge of these growth factors that the operator can truly "see" what is going on in the treatment plant
Lesson 3 - Population Dynamics

Slide #

#1 & #2

Title and Credit Slides

#3

Population Dynamics - The changes that occur within the microorganism population as a result of environmental factors

#4

Predominance - The organisms that get the most to eat and do it the fastest and most efficiently will predominate

Efficiency

#5

Aerobic metabolism is more efficient; aerobic organisms will predominate when free oxygen is present

#6, #7, #8

Anaerobic metabolism uses bound oxygen; less efficient

Competition

#9

If there is one group of organisms alone they will use food at a certain efficiency

#10

If a second group is present the one that is the most efficient at converting the same food will predominate

#11

If two organisms are present the one that converts food the fastest will predominate

#12

If two groups use the same food but one is much more efficient the second may get left out all together

#13

unless they can switch to a second choice of food. Then they will survive also.

Growth Pressures

#14

Temperature

A shift in temperature may favor the increase in one type of organism

I-CB-9
pH
A shift in pH may favor the increase in one or the decrease in another type of organism

Dissolved Oxygen
If dissolved oxygen is lost the population will shift from aerobes and facultative to facultative and anaerobes

Toxics
The presence of toxic materials may stop the growth of some types and inhibit others; some may not be greatly affected

Fixed Film and Suspended growth systems are both affected by environmental growth pressures

The Aquatic Food Chain
Bacteria are at the bottom, followed by protozoa, fungi, algae, insect larvae and higher aquatic organisms

The protozoa vs bacteria growth curve
Can be used to assess the condition of the bacteria in activated sludge
As food decrease protozoa appear in the following order: amoeba, flagellates, free-swimming ciliates, stalked ciliates, and rotifers

The algae/bacteria symbiosis
Algae use energy from the sun, carbon dioxide and minerals from the bacteria to produce oxygen; the bacteria use the oxygen from the algae and organic material

Primary/Secondary Predominance
Primary Predominance - Hard to digest foods can be metabolized by bacteria that have been acclimated to that special food source
Secondary Predominance -
After the primary organisms have
partially metabolized the material
the less specialized organisms
can deal with the material

Dynamic Equilibrium
Keeping a changing system in balance

Energy in must equal the energy
going out of the system

Review of Population Dynamics
CONCEPTS OF BIOLOGICAL TREATMENT

Answers to Worksheet 1 - The Microorganisms

1. We use the term "bugs" to mean all of the [microorganisms] in the biological treatment system.

2. The primary objective of wastewater treatment is to:
   prevent the degradation of the receiving stream by reducing the amount of organic material discharged to it.

3. When organic material is allowed to enter a stream the natural biological activity in the stream depletes the [oxygen].

4. In biological treatment processes, with the exception of anaerobic digestion, the bugs or microorganisms are supplied with [air (oxygen)] so they can consume the food in a controlled environment.

5. The only drawback to the bugs eating and eating is that they create excess [solids (cells)].

6. Name the five major groups of bugs typically found in biological treatment systems:
   - bacteria
   - protozoa
   - viruses
   - algae
   - fungi

7. The microorganism which is responsible for the majority of the stabilization activity is the [bacteria].

8. On the average bacteria are about [1 micron] wide.

9. The three structural shapes of bacteria are:
   - rod (bacillus)
   - spheres (cocci)
   - spirals
10. The slimy, gummy coat around some bacteria is called the capsule.

11. The protozoa are a group of microscopic animals.

12. The four major groups of protozoa found in biological treatment systems are:
   - amoeba
   - ciliates
   - flagellates
   - suctoria

13. Amoeba move about with the use of pseudopods or false feet.

14. The two types of ciliates are the free-swimming and the stalked.

15. The Mastigophora move about with the use of flagella.

16. The suctoria resemble the ciliates except they have tentacles instead of cilia.

17. The viruses are strictly parasitic because they have no enzyme systems.

18. The algae are true photosynthetic plants.

19. Four types of "higher" animal forms are frequently found in and around biological systems. These include:
   - insect larvae
   - rotifers
   - worms
   - snails
CONCEPTS OF BIOLOGICAL TREATMENT

Answers to Worksheet 2 - Environmental Factors

1. The main "objective" of the microorganisms in the treatment system is to obtain food and energy for maintenance and reproduction.

2. The environmental factors that cause the microorganisms to react in some way are often called environmental pressures.

3. The food materials available to the microorganisms are mostly organic materials.

4. The phase of a bacterial growth curve in which the bacteria are increasing at a maximum rate is called the log growth phase.

5. The phase of the growth curve in which food is not available and the bacteria are utilizing energy stored within their own cells is called the endogenous phase.

6. The amount of liquid entering the system is called the hydraulic load.

7. Most organisms in the biological treatment processes use free dissolved oxygen. These are called aerobic organisms.

8. At times bacteria carry out anaerobic respiration in which they use chemically bound oxygen.

9. Many bacteria in biological systems are capable of using either aerobic or anaerobic respiration. These organisms are said to be facultative.

10. Microorganisms that thrive at about 35 degrees Celsius are called mesophilic organisms.

11. Microorganisms that prefer relatively high temperatures are called thermophilic organisms.

12. Most microorganisms in the biological treatment systems do well at a pH in the range of 6.5 to 8.5.

13. One group of microorganisms tend to prefer lower pH and high carbohydrate conditions. These are the fungi.

14. Large amounts of toxic or poisonous materials could actually kill (destroy) all the microorganisms in the system.
CONCEPTS OF BIOLOGICAL TREATMENT

Answers to Worksheet 3 - Population Dynamics

1. The term population dynamics is used to describe the continuous fluctuations that occur with the many types of microorganisms in the system.

2. Predominance is established by the microorganisms that derive energy from the available food most efficiently.

3. The process of converting energy in the organic food to usable energy is called respiration.

4. As a result of the energy derived aerobically from the organics, new cells are produced along with two by-products: carbon dioxide and water.

5. Anaerobic respiration produces different by-products depending on the source of bound oxygen used. These are:

   for nitrate bound oxygen = nitrogen gas (N₂)
   for sulfate bound oxygen = hydrogen sulfide (H₂S)
   for carbon dioxide oxygen = methane (CH₄)

6. Anaerobic respiration will derive only about 15 percent as much energy from the same organics as from aerobic respiration.

7. The food (organics) provides energy and also raw (building) material needed to produce new cells.

8. Efficiency of converting food to usable energy can be accomplished either by converting organics rapidly or completely.

9. Name three growth pressures other than oxygen and food that can affect population predominance.

   temperature, pH, sunlight, salinity, toxic materials

10. At times one organism will prey upon a certain type of organism and at the same time be preyed upon by a third type. This is an example of the food chain.

11. Organisms at the bottom of the food chain are typically smaller, but in total have a larger mass.
12. **Protozoa** can be used to observe the conditions of the bacterial population because the protozoa feed on the **bacteria**.

13. During photosynthesis the algae utilize **sunlight**, **inorganics**, and **carbon dioxide** to produce new algae cells and oxygen.

14. The algae are provided with the inorganics and carbon dioxide by the **bacteria** in the system.

15. A healthy population of bacteria can be developed to act as **primary** predominance on almost any type of waste material.
CONCEPTS OF BIOLOGICAL TREATMENT

Final Quiz

MATCHING: Choose the best answer(s) for each of the following questions and place an "X" in the appropriate space.

1. When microorganisms in a treatment plant come into contact with food (organic material), they:
   - a. eat and reproduce
   - b. become aerobic
   - c. become anaerobic
   - d. die
   - e. settle

2. Which of the following characteristics does NOT describe logarithmic growth:
   - a. microorganisms grow and multiply at their maximum rate
   - b. food is in excess
   - c. continues until food begins to be used up
   - d. common when there is a high F/M ratio (food:microorganism)
   - e. slow growth rate

3. Declining growth occurs when:
   - a. food is almost used up and growth rate slows down
   - b. food is in excess
   - c. microorganisms grow and multiply at their maximum rate
   - d. microorganisms are in endogenous respiration
   - e. there is a high F/M ratio (food:microorganism)

4. Endogenous growth phase occurs when:
   - a. there is a low F/M ratio
   - b. food is in excess
   - c. microorganisms grow and multiply at their maximum rate
   - d. there is a high F/M ratio
   - e. microorganisms use up food reserves that is stored inside their cells

I-CB-17
5. "Hydraulic load" refers to:
   ____ a. the amount of food entering a system
   ____ b. the amount of water entering a system
   ____ c. the weight of water in a system
   ____ d. the weight of organic material in a system
   ____ e. the length of pipe in the collection system

6. Some bacteria obtain the oxygen they need from chemically bound compounds, and in fact, can't grow when free oxygen is present. These organisms are called:
   ____ a. anaerobes
   ____ b. aerobes
   ____ c. microbes
   ____ d. facultative
   ____ e. filaments

7. Temperature is a growth pressure. As a general rule, as temperature goes up:
   ____ a. growth rate slows down
   ____ b. growth rate speeds up
   ____ c. growth rate stays the same
   ____ d. pH goes up
   ____ e. pH goes down

8. Microorganisms that grow best at high temperatures are called:
   ____ a. thermophilic
   ____ b. mesophilic
   ____ c. psychrophillic
   ____ d. anaerobic
   ____ e. aerobic

9. pH is a term which describes how acidic or basic a solution is. pH 7 is considered:
   ____ a. acidic
   ____ b. basic
   ____ c. neutral
10. As waste materials break down in a stream:
   ___ a. temperature goes down
   ___ b. fish reproduce
   ___ c. dissolved oxygen is consumed
   ___ d. temperature goes up
   ___ e. dissolved oxygen goes up

11. What group of microorganisms do the most work in a secondary treatment plant?
   ___ a. viruses
   ___ b. fungi
   ___ c. worms
   ___ d. bacteria
   ___ e. protozoa

12. Bacteria are:
    ___ a. single-celled
    ___ b. multi-celled
    ___ c. always aerobic
    ___ d. visible to the naked eye
    ___ e. always the same shape

13. Rod-shaped bacteria are also called:
    ___ a. coccus
    ___ b. spirillum
    ___ c. bacillus
    ___ d. vibrio
    ___ e. sarcina

14. Protozoa are:
    ___ a. usually about a hundred times bigger than bacteria
    ___ b. about the same size as bacteria
    ___ c. photosynthetic
    ___ d. always non-motile
    ___ e. responsible for doing the most work in secondary treatment
15. Which of the following does NOT apply to free-swimming ciliates:
   a. move with flagella
   b. usually ovoid to pear-shaped
   c. single-celled
   d. have fine "hair-like" appendages for locomotion
   e. are larger than bacteria

16. Which of the following does NOT apply to stalked ciliates:
   a. usually attached to something in the water (substrate)
   b. are single-celled
   c. may be colonial
   d. are common in biological treatment systems
   e. use flagella for locomotion

17. Which of the following does not apply to flagellates:
   a. also called Mastigophora
   b. are usually ovoid to pear-shaped
   c. single-celled
   d. use flagella for locomotion
   e. are covered with fine "hair-like" appendages for feeding

18. Which of the following characteristics does NOT apply to a virus:
   a. are strictly parasitic and lack the metabolic systems necessary to
      obtain energy on their own, or even reproduce by themselves.
   b. they steal energy and raw materials of their host.
   c. are about the same size as bacteria.
   d. are a hundred to a thousand times smaller than bacteria.
   e. they do very little work in secondary treatment

19. When one type of organism derives energy more efficiently than another
   type
   a. anaerobic respiration is occurring
   b. aerobic respiration is occurring
   c. the pH is too high
   d. predominance will occur
   e. one will die completely

I-CB-20
20. By-products of anaerobic respiration include
   __ a. nitrogen gas
   __ b. nitrate
   __ c. hydrogen sulfide
   __ d. methane gas
   __ e. all of the above

21. Anaerobic respiration will derive about ___% as much energy from the same organic material as aerobic respiration.
   __ a. 5
   __ b. 10
   __ c. 15
   __ d. 25
   __ e. 50

22. Which of the following are environmental pressures that can cause predominance to shift?
   __ a. temperature
   __ b. pH
   __ c. sunlight
   __ d. salinity
   __ e. toxic materials

23. In the prey-predator relationship known as the food chain:
   __ a. those at the top of the chain are the ultimate prey.
   __ b. protozoa are lower than bacteria and algae.
   __ c. organisms at the bottom are typically smaller.
   __ d. those at the top are dependent upon those at the bottom.
   __ e. the mass of those at the bottom is greater than that of those at the top.

24. Which of the following is NOT true about the protozoa population dynamics in activated sludge?
   __ a. protozoa feed on bacteria
   __ b. amoeba and flagellates are found under high food availability
   __ c. rotifers are found at the same time that the bacterial population is maximum.
   __ d. stalked-ciliates begin to predominate as the food supply begins to diminish.
   __ e. free-swimming ciliates predominate at the same time as the bacteria are at maximum.
25. In the algae-bacteria inter-relationship
   a. algae use sunlight for energy
   b. algae produce oxygen
   c. bacteria use organics
   d. bacteria provide dissolved inorganics, and carbon dioxide
   e. bacteria use carbon dioxide
CONCEPTS OF BIOLOGICAL TREATMENT

Answers to Final Quiz

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