Arba Minch Water Technology Institute

Course title; **Wastewater Microbiology**

Course code; **WSEE-2093**

Target groups; **2nd year Water Supply and Environmental Engineering students**

Instructor; **Tizita Debebe**

Course Objectives

- The course provides basic knowledge of waste microbiology and possible methods of treatment, basic parameters, processes and control methods.

Teaching methods and Assessment

- Lectures. Laboratory and home study
- 50%Continuous assessment (assignments, reports, lab. , quiz)
- 50%Final exam

Course contents

- Water and Environment related Biology
- Cell biology
- Microbiology
- Waste microbiology, type of microbes existing in liquid wastes, possible methods of treatments

1. General

Purpose

- to provide a fundamental background on the relationship between microbes, wastewater, and wastewater treatment.
- It will explain why we are concerned about microbes in wastewater, what role do they play in wastewater treatment

What is Microbiology?

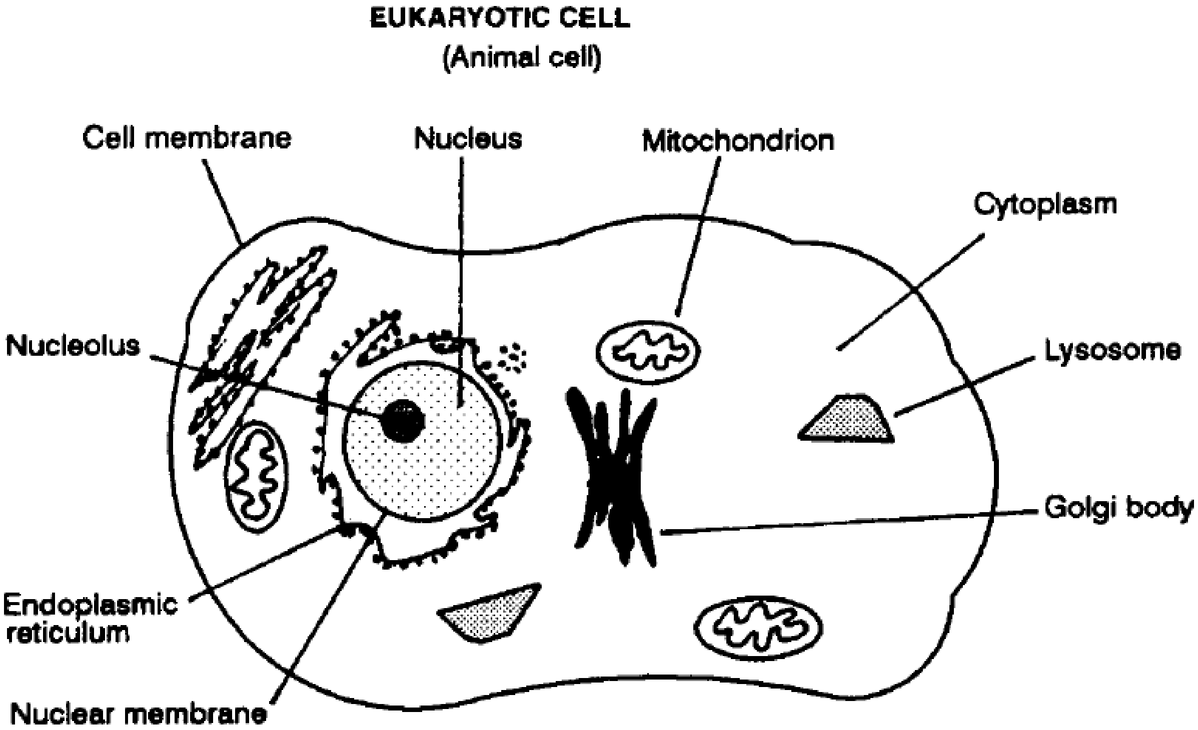
- Micro - too small to be seen with the naked eye
- Bio - life
- logy - study of

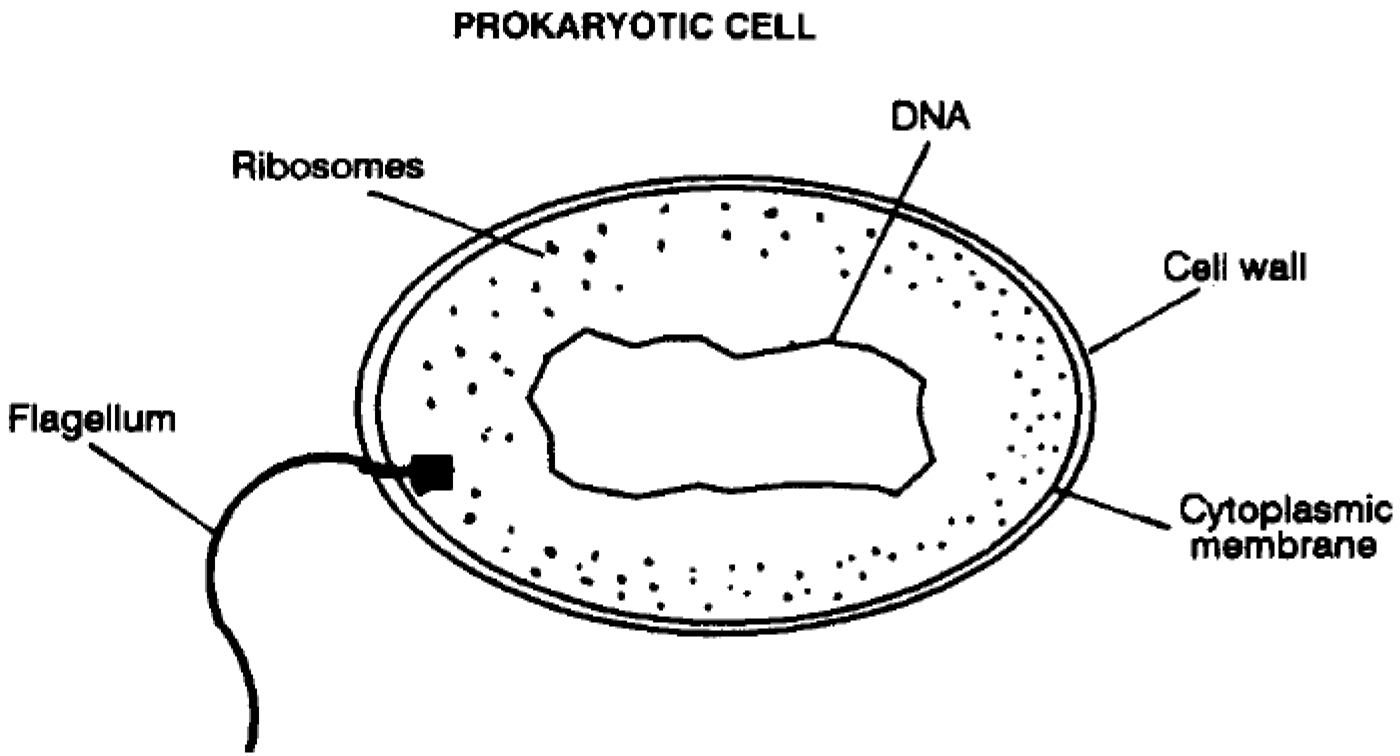
The study of too small organisms which can not be seen with the naked eye.

Classification System

- The three domains of life are bacteria, archaea, and eukarya
  1. Bacteria
     - Unicellular prokaryotes with cell wall containing peptidoglycan
  2. Archaea
     - Unicellular prokaryotes with no peptidoglycan in cell wall
  3. Eukarya
     - Protozoa
     - Fungi
     - Plants
     - Animals

Cell Structure




PROKARYOTIC CELL
DNA
Ribosomes
Cell wall
Flagellum
Cytoplasmic
membrane

Organisms included in the study of Microbiology

1. Bacteria	Bacteriology
2. Protozoans	Protozoology
3. Algae	Phycology
4. Parasites	Parasitology
5. Yeasts and Molds	
– Fungi	Mycology
6. Viruses	Virology

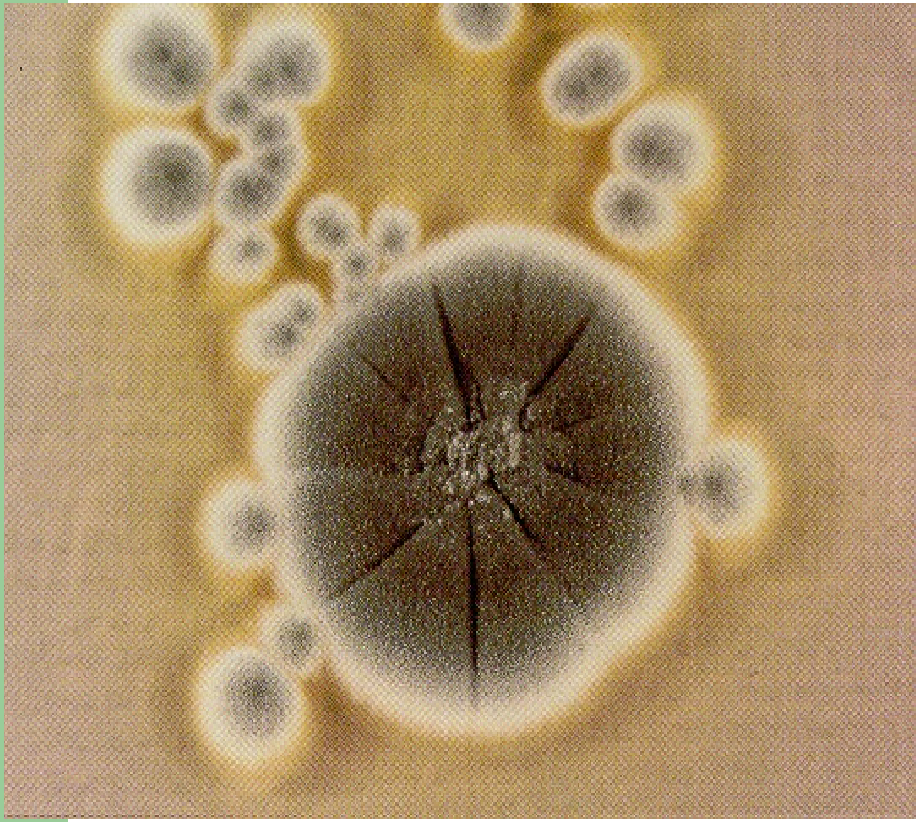
Bacteria - what comes to mind?

- Diseases
- Infections
- Epidemics
- Food Spoilage
- Only 1% of all known bacteria cause human diseases
- About 4% of all known bacteria cause plant diseases
- 95% of known bacteria are non-pathogens

Microbes Benefit Humans

1. ***Bacteria are primary decomposers*** - recycle nutrients back into the environment (sewage treatment plants)
2. ***Microbes produce various food products***
   - cheese, pickles, sauerkraut, green olives
   - yogurt, soy sauce, vinegar, bread
   - Beer, Wine, Alcohol

3.Microbes are used to produce Antibiotics



- Penicillin
- Mold
  - *Penicillium notatum*

1928 Alexander Fleming

4.Insect Pest Control

- Using bacteria to control the growth of insects
- *Bacillus thuringiensis*
  - caterpillars
  - bollworms
  - corn borers

5. Bioremediation

- Using microbes to clean up pollutants and toxic wastes

- 2 Genera
  - *Pseudomonas sp.*
  - *Bacillus sp.*

2. Microbial metabolism and growth

Enzymes?

- are protein molecules that serve as catalysts of biochemical reactions in animal, plant, and microbial cells.

- Enzymes do not undergo structural changes after participating in chemical reactions and can be used repeatedly

- lower the activation energy and increase the rate of biochemical reactions

Enzyme kinetics

- is the study of the chemical reactions that are catalysed by enzymes.
- In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction is investigated.

Important factors controlling enzymatic reactions are:

- substrate concentration
- pH
- temperature
- ionic strength
- presence of toxicants

Effect of Inhibitors on Enzyme Activity

- A wide range of toxicants are commonly found in industrial and municipal wastewater treatment plants.

- These inhibitors decrease the activity of enzyme-catalyzed reactions.

Microbial Metabolism

- Metabolism is the sum of biochemical transformations that includes interrelated catabolic and anabolic reactions.

- can also refer to all chemical reactions that occur in living organisms

Metabolism

Catabolism

- ✓breaks down organic matter and harvests energy by way of cellular respiration

Anabolism

- ✓uses energy to construct components of cells such as proteins and nucleic acids.

- Catabolic reactions are exergonic and release energy derived from organic and inorganic compounds

- Anabolic reactions (i.e., biosynthetic) are endergonic: they use the energy and chemical intermediates provided by catabolic reactions for biosynthesis of new molecules, cell maintenance, and growth

- Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy that will not occur by themselves, by coupling them to spontaneous reactions that release energy.

- Enzymes act as catalysts that allow the reactions to proceed more rapidly.

Microbial Growth Kinetics

- Growth of a microbial population is defined as an increase in numbers or an increase in microbial mass.

- Growth rate is the increase in microbial cell numbers or mass per unit time.

MICROBIAL GROWTH KINETICS

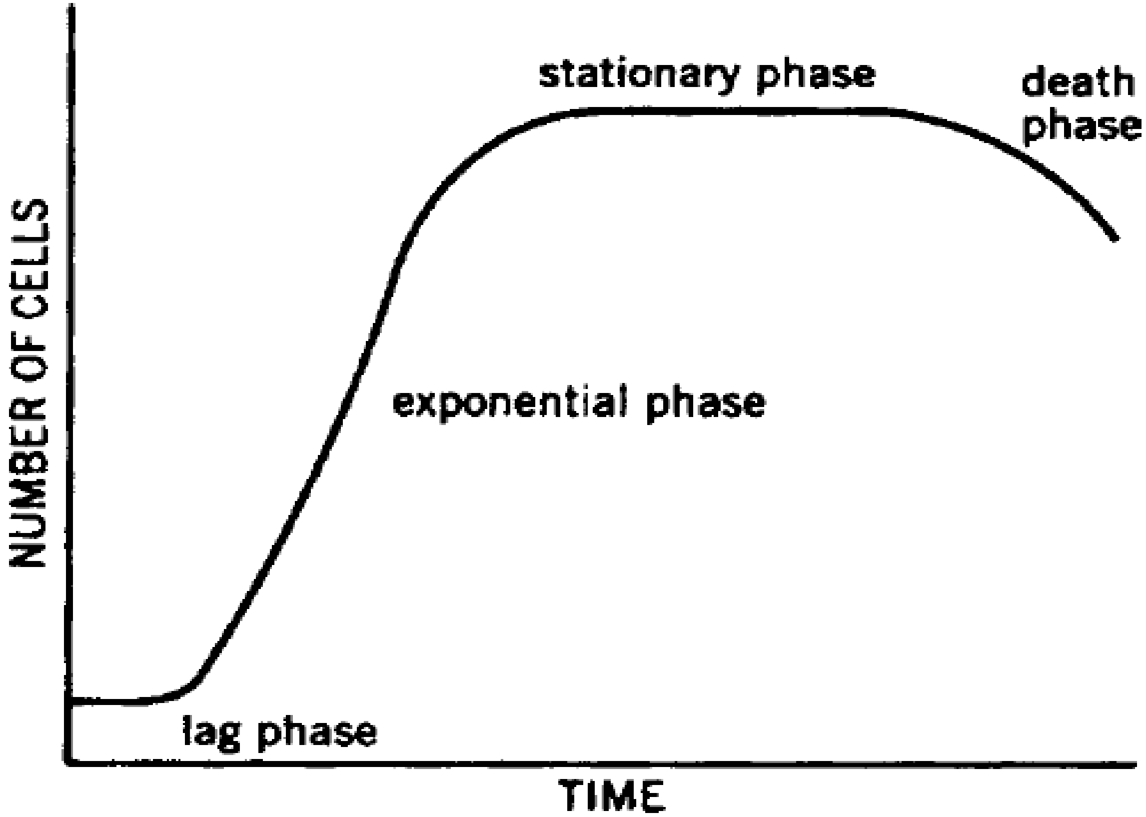
- Microbial populations can grow as

1. batch cultures (closed systems) or
2. Continuous cultures (open systems) (Marison, 1988a).

Batch Cultures

- When a suitable medium is inoculated with cells, the growth of the microbial population follows the growth curve displayed in Figure below which shows four distinct phases.

Batch Cultures



Batch Cultures

- Lag Phase:

is a period of cell adjustment to the new environment.

Cells are involved in the synthesis of biochemicals and undergo enlargement

Batch Cultures

- Exponential Growth Phase (Log Phase):

The number of cells increase exponentially during the log phase.

The growth follows a geometric progression

2^0 2^1 2^2 2^n

Batch Cultures

$$X_t = X_0 e^{\mu t}$$

- where

μ: specific growth rate (h^{-1})

Xt: cell biomass or numbers after time t,

X_0: initial number or biomass of cells.

Batch Cultures

- Using the natural logarithms on both sides of above Eq. we obtain:

$$\ln X_t = \ln X_0 + \mu t$$

μ is given by

$$\mu = \frac{\ln X_t - \ln X_0}{t}$$

Batch Cultures

If n is the number of population doublings (i.e., number of generations) after time t, the doubling time td is given by

$$t_d = \frac{t}{n}$$

Batch Cultures

- μ is related to the doubling time t_d by:

$$\mu = \frac{\ln 2}{t_\mathrm{d}} = \frac{0.693}{t_\mathrm{d}}$$

Batch Cultures

- Stationary Phase:

because

- microorganisms cannot grow indefinitely, mainly because of lack of nutrients and electron acceptors, and
- the production and the accumulation of toxic metabolites.

Batch Cultures

- Death Phase:

During this phase, the death (decay) rate of the microbial population is higher than the growth rate.

Cell death may be accompanied by cell lysis.

Continuous Culture of Microorganisms

- Maintenance of microbial cultures at the exponential growth phase over a long period of time can be achieved by:

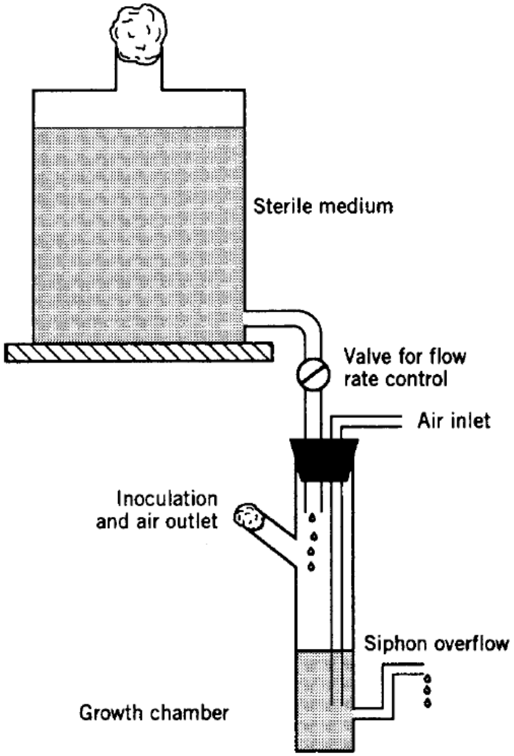
- growing continuously the cells in a completely mixed reactor in which a constant volume is maintained.

Continuous Culture of Microorganisms

- most commonly used device is the chemostat
- which is essentially a complete-mix bioreactor without recycle.

- In addition to the flow rate of growth-limiting substrate, environmental parameters such as oxygen level, temperature, and pH are also controlled.

Continuous Culture of Microorganisms



Continuous Culture of Microorganisms

- The substrate is added continuously at a flow rate Q to a reactor with a volume V containing concentration X of microorganisms.

- The dilution rate D, the reciprocal of the hydraulic retention time t, is given by

$$D = \frac{Q}{V} = \frac{1}{t}$$

Continuous Culture of Microorganisms

- where D = dilution rate ($time^{-1}$),

  V=reactor volume (L),

  Q = flow rate of substrate S (L/time),

and t = time.

In continuous-flow reactors, microbial growth is described by:

$$\frac{dX}{dt} = \mu X - DX = X(\mu - D)$$

$$X_t = X_0 e^{(\mu - D)t}$$

Continuous Culture of Microorganisms

- At $D > \mu_{max}$, we observe a decrease in cell concentration and a washout of the population.

- Cell washout starts at the critical dilution rate Dc , which is approximately equal to μ_{max}.

Continuous Culture of Microorganisms

The mass balance for X is given by:

$$V\frac{dX}{dt} = \mu XV - QX$$

$$= \frac{\mu_{\max} S}{K_s + S} XV - QX$$

Continuous Culture of Microorganisms

- At steady state:

$$\frac{dX}{dt} = 0 \rightarrow \mu = D = \frac{Q}{V} = \frac{\mu_{\max} S}{K_s + S}$$

Continuous Culture of Microorganisms

- At steady state, the substrate concentration S and the cell concentration X in the reactor are given by:

$$S = K_s \frac{D}{\mu_{max} - D}$$

$$X = Y(S_i - S_e)$$

Continuous Culture of Microorganisms

where

Y= growth yield, S_i= influent substrate concentration, and

Se = effluent substrate Concentration

Other Kinetic Parameters

- Three important parameters in microbial growth kinetics:

I. growth yield Y,
II. Specific growth rate μ, and
III. specific substrate uptake rate q.

Other Kinetic Parameters

- The rate of increase of microorganisms in a culture (dX/dt) is proportional to the rate of
- substrate uptake/removal (dS/dt) by microbial cells:

$$\frac{dX}{dt} = Y \frac{dS}{dt}$$

Other Kinetic Parameters

where

- Y = growth yield coefficient expressed as mg cells formed per mg of substrate used,
- dX/dt = rate of increase in microorganism concentration (mg/L/day), and
- dS/dt = rate of substrate removal (mg/L/day).

Other Kinetic Parameters

- A more simplified equation showing the relationship between the three parameters is the following:

$$\mu = Yq$$

Other Kinetic Parameters

Where

 μ=specific growth rate (time-1),

Y =growth yield (mg cells formed per mg of substrate removed), and

q=substrate uptake rate (mg/L/day).

Growth Yield

- is the amount of biomass formed per unit of amount of substrate removed.
- It reflects the efficiency of conversion of substrate to cell material.
- The yield coefficient Y is obtained as

$$Y = \frac{X - X_0}{S_0 - S}$$

Growth Yield

- where S_0 and S =initial and final substrate concentrations, respectively (mg/L or mol/L),
- X_0 and X =initial and final microbial concentrations, respectively.

Growth Yield

- particularly in wastewater, there is a wide range of microorganisms, few of which are in the logarithmic phase.

- Many are in the stationary or in the declining phase of growth.
- The growth yield Y must be corrected for the amount of cell decay occurring during the declining phase of growth.

Growth Yield

- true growth yield coefficient

$$\mu = Yq - k_\mathrm{d}$$

- where kd is the endogenous decay coefficient (day-1)

Specific Substrate Uptake Rate q.

- The specific substrate uptake (removal) is given by q:

$$q = dS/dt/X$$

- where q (time-1) is given by the Monod's equation:

$$q = q_{\max} \frac{[S]}{K_s + [S]}$$

Specific Growth Rate μ

- This is given by:

$$\mu = \frac{dX/dt}{X}$$

Where

μ (day-1) is given by Monod’s equation:

$$\mu = \mu_{max} \frac{[S]}{K_s + [S]}$$

Specific Growth Rate μ

- The in situ specific growth rate of bacteria in wastewater was measured using the labeled thymidine growth assay (thymidine is a precursor of DNA in cells).
- In an aerobic tank, the specific growth rate m was 0.5 d21 (doubling time td ¼ 1.4 d) whereas in an anaerobic tank m was equal to 0.2 d-1 (td ¼ 3.9 d)

(Pollard and Greenfield, 1997

Specific Growth Rate μ

- In waste treatment, the reciprocal of μ is the biological solid retention time θ_c , that is

$$\mu = 1/\theta_c$$

$$1/\theta_c = Yq - k_d$$

Physical and Chemical Factors Affecting Microbial Growth

- Substrate Concentration
- Temperature
- pH

Biological treatment of wastewater occurs generally at neutral

-bacterial growth is around 7

- Oxygen Level

Physical and Chemical Factors Affecting Microbial Growth

Microorganisms can grow in the presence or in the absence of oxygen.

- There are divided into

1. strict aerobes,
2. facultative anaerobes (can grow in the

presence or in the absence of oxygen), and strict anaerobes.

3. PATHOGENS AND PARASITES IN DOMESTIC WASTEWATER

ELEMENTS OF EPIDEMIOLOGY

- Epidemiology is the study of the spread of infectious diseases in populations.

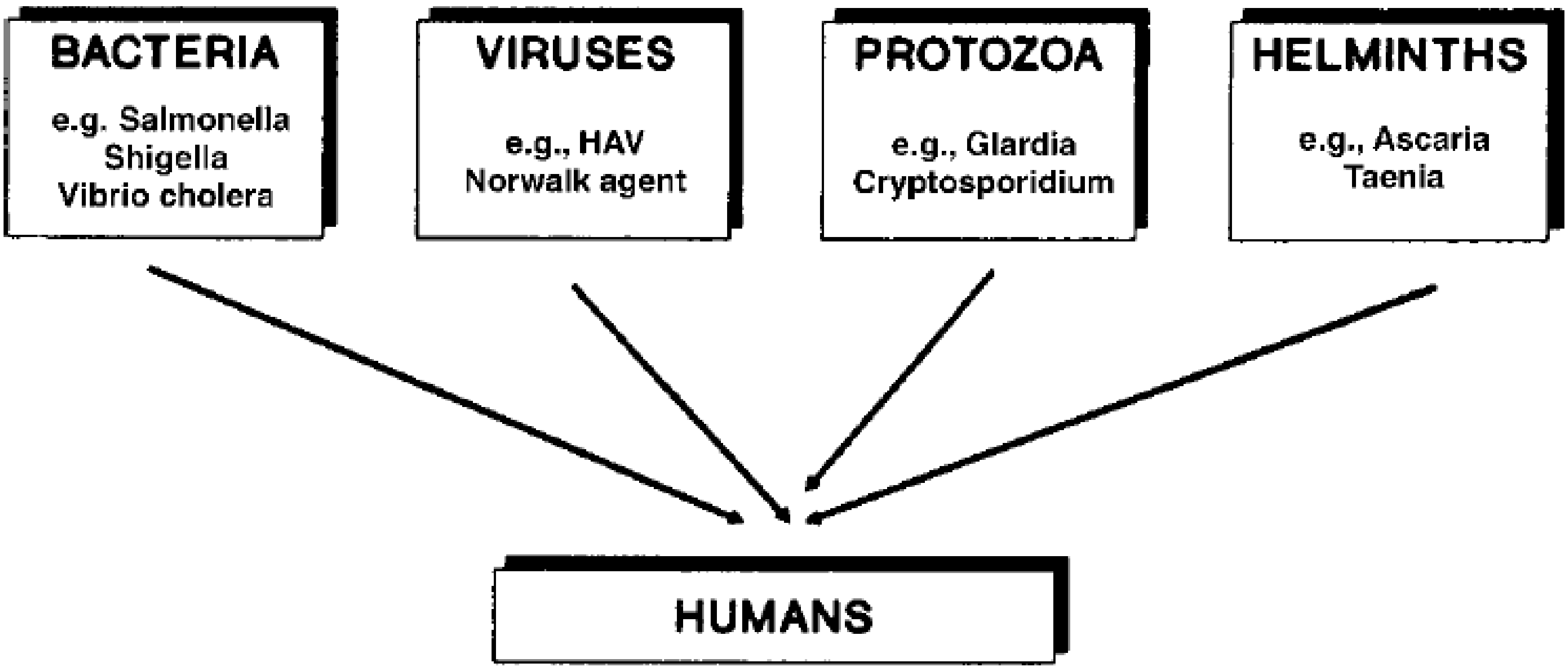
- Infectious diseases are those that can be spread from one host to another.

- Incidence of a disease is the number of individuals with the disease in a population, whereas prevalence is the percentage of individuals with the disease at a given time.

- Pathogenicity is the ability of an infectious agent to cause disease and injure the host.

- The development of the disease depends on various factors,
  - including infectious dose,
  - pathogenicity, and host and environmental factors.
- Some organisms, however, are opportunistic pathogens that cause disease only in compromised individuals.

- Type of Infectious Agent. Several infectious organisms may cause diseases in humans. These agents include bacteria, fungi, protozoa, metazoa (helminths), rickettsiae, and viruses (Fig. below).


BACTERIA
e.g. Salmonella
Shigella
Vibrio cholera
VIRUSES
e.g., HAV
Norwalk agent
PROTOZOA
e.g., Glardia
Cryptosporidium
HELMINTHS
e.g., Ascaria
Taenia
HUMANS

Mode of Transmission:

- Transmission involves the transport of an infectious agent from the reservoir to the host.

Person-to-Person Transmission

e.g AIDS, syphilis, gonorrhea, herpes

Waterborne Transmission,

e.g cholera

- Foodborne Transmission
- Airborne Transmissions
- Vector-Borne Transmission

Pathogens And Parasites Found In Domestic Wastewater

- Several pathogenic microorganisms and parasites are commonly found in domestic wastewater as well as in effluents from wastewater treatment plants.

- The three categories of pathogens encountered in the environment are (Leclerc et al., 2002):

Pathogens And Parasites Found In Domestic Wastewater

1. **Bacterial pathogens:**

- pathogens (e.g., Salmonella, Shigella) are enteric bacteria.
- Others (e.g., Legionella, Mycobacterium avium, Aeromonas) are indigenous aquatic bacteria.

Pathogens And Parasites Found In Domestic Wastewater

- Wastewater bacteria have been characterized and belong to the following groups (Dott and Kampfer, 1988):

Gram-negative:- facultatively anaerobic bacteria: e.g., Aeromonas, Plesiomonas, Vibrio, Enterobacter, Escherichia, Klebsiella, and Shigella.

Pathogens And Parasites Found In Domestic Wastewater

Gram-negative aerobic bacteria: e.g., Pseudomonas, Alcaligenes, Flavobacterium, Acinetobacter

Gram-positive spore forming bacteria: e.g., Bacillus spp

Nonspore-forming gram-positive bacteria: e.g., Arthrobacter, Corynebacterium, Rhodococcus.

Pathogens And Parasites Found In Domestic Wastewater

Bacterial Agent	Major Disease	Major Reservoir	Principal Site Affected
Salmonella typhi	Typhoid fever	Human feces	Gastrointestinal tract
Salmonella paratyphi	Paratyphoid fever	Human feces	Gastrointestinal tract
Shigella	Bacillary dysentery	Human feces	Lower intestine
Vibrio cholerae	Cholera	Human feces	Gastrointestinal tract
Pathogenic *E. coli*	Gastroenteritis	Human feces	Gastrointestinal tract
	Hemolytic uremic syndrome		

Pathogens And Parasites Found In Domestic Wastewater

2. **Viral pathogens:**

These are also released into aquatic environments but are unable to multiply outside their host cells.

- Water and wastewater may become contaminated by approximately 140 types of enteric viruses.

Pathogens And Parasites Found In Domestic Wastewater

3. Protozoan Parasites

Most of the protozoan parasites produce cysts, which are able to survive outside their host under adverse environmental conditions.

- The major waterborne pathogenic protozoa affecting humans are:
  - Giardia
  - Cryptosporidium.
  - Cyclospora

3. MICROBIAL INDICATORS OF FECAL CONTAMINATION

MICROBIAL INDICATORS OF FECAL CONTAMINATION

- The direct detection of pathogenic bacteria and viruses, and cysts of protozoan parasites requires costly and time-consuming procedures.

- Indicator bacteria are then very important to detect the pollution level of the waste water.

- various microorganisms have been proposed and used for indicating the occurrence of fecal contamination, treatment efficiency in water and wastewater treatment plants, The criteria for an ideal indicator organism are:

It should be :-

- one of the intestinal micro flora of warm-blooded animals.
- present when pathogens are present, and absent in uncontaminated samples

- present in greater numbers than the pathogen.
- equally resistant as the pathogen to environmental insults and to disinfection in water and wastewater treatment plants.
- not multiply in the environment.
- detectable by means of easy, rapid, and inexpensive methods.
- nonpathogenic

microbial indicators

1. Total Coliforms

- belongs to the family enterobacteriaceae and includes the aerobic and facultative anaerobic, gram-negative, nonspore forming, rod-shaped bacteria that ferment lactose with gas production within 48 hours at 35 ^{0}C

This group includes

- Escherichia coli
- Enterobacter
- Klebsiella
- Citrobacter

- These indicators are useful for determining the quality of potable water, shellfish-harvesting waters, and recreational waters.

- They are less sensitive, however, than viruses or protozoan cysts to environmental factors and to disinfection.

2. Fecal Coliforms

- Fecal coliforms or thermotolerant coliforms include all coliforms that can ferment lactose at 44.5 ^{0}C.

- group comprises bacteria such as Escherichia coli or Klebsiella pneumonae.

- presence of fecal coliforms indicates the presence of fecal material from warm-blooded animals.
- but their usefulness as indicators of protozoan or viral contamination is limited.
- much less resistant to disinfection than viruses or protozoan cysts.

3. Fecal Streptococci

- This group comprises Streptococcus faecalis, S. bovis, S. equinus, and S. avium.
- commonly inhabit the intestinal tract of humans and warm-blooded animals,
- they are used to detect fecal contamination in water.

- Members of this group survive longer than other bacterial indicators, but do not reproduce in the environment

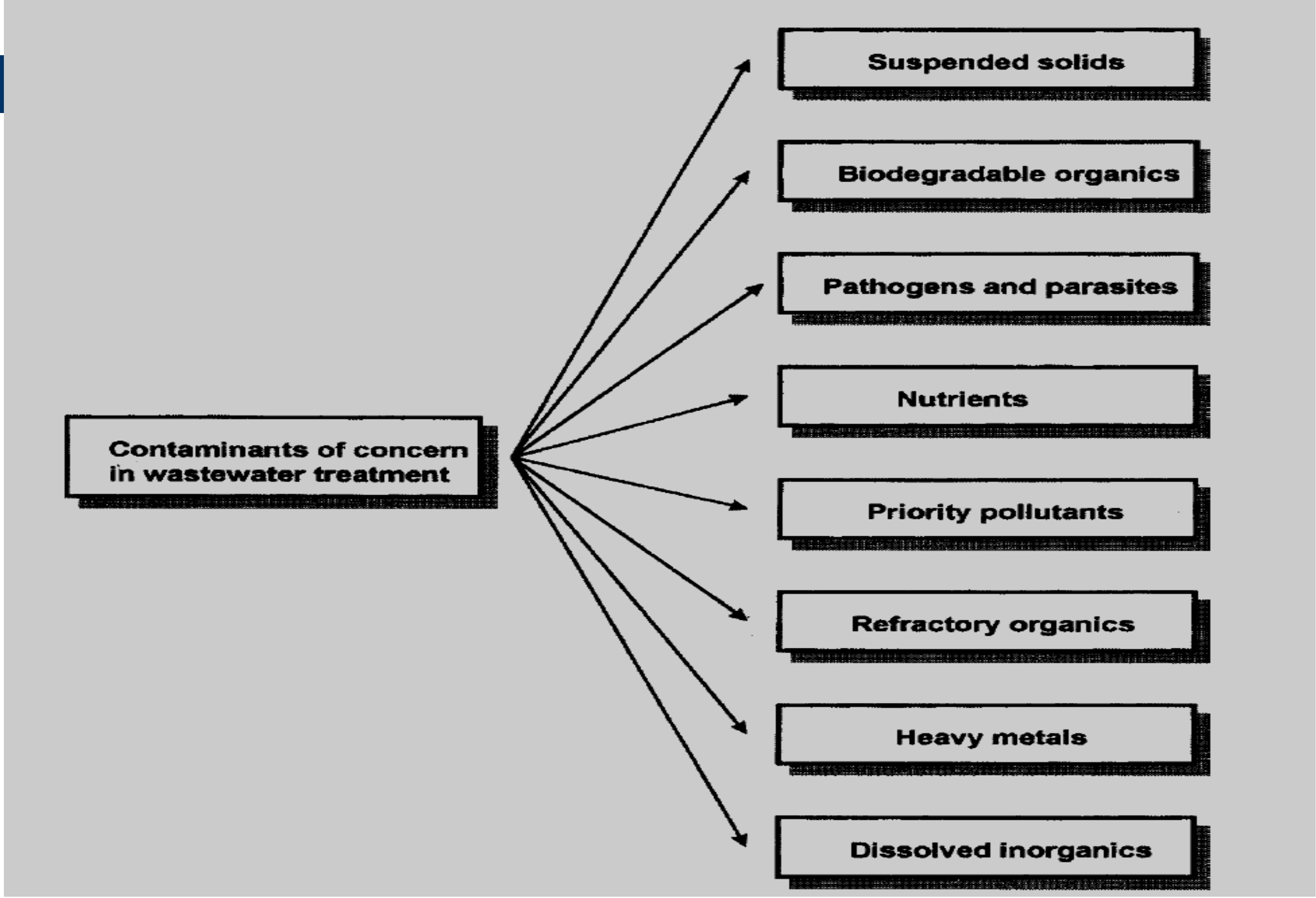
- A subgroup of the fecal streptococci group, the enterococci (S. faecalis and S. faecium), has been suggested as useful for indicating the presence of viruses,

4. INTRODUCTION TO WASTEWATER TREATMENT

Introduction

- Increasing awareness of the role of microorganisms in diseases led to an enhanced demand for wastewater treatment.

❖The major contaminants found in wastewater are



- Research efforts are now being focused on the removal of:
  - nutrients (N, P)
  - odors
  - volatile organic compounds
  - metals
  - toxic organics after their passage through wastewater treatment plants

Composition of Domestic Wastewater

- combination of human and animal excreta (feces and urine) and gray water resulting from washing, bathing, and cooking, composed mainly of :
  - proteins (40–60 percent)
  - Carbohydrates (25–50 percent)
  - fats and oils (10 percent)
  - urea derived from urine

Composition of Domestic Wastewater

- and a large number of trace organic compounds that include pesticides, surfactants, phenols, and priority pollutants.

- In domestic wastewaters, organic matter occurs as dissolved organic carbon (DOC) and particulate organic carbon (POC).

Composition of Domestic Wastewater

- Three main tests are used for the determination of organic matter in wastewater

i. BOD

ii. TOC

iii. COD

COMPOSITION OF DOMESTIC WASTEWATER

i. BOD

- Biochemical oxygen demand (BOD) is the amount of dissolved oxygen (DO) consumed by microorganisms for the biochemical oxidation of organic (carbonaceous BOD) and inorganic matter (autotrophic or nitrogenous BOD).

Composition of Domestic Wastewater

- The BOD test was originally developed to predict the effect of wastewater on receiving streams and determine their capacity to assimilate organic matter (Gaudy, 1972; Gaudy and Gaudy, 1988).

COMPOSITION OF DOMESTIC WASTEWATER

- Dissolved oxygen concentration is determined at time 0 and after five-day incubation, by means of an oxygen electrode, chemical procedures (e.g., the Winkler test), or a manometric BOD apparatus (Hammer,1986).

Composition of Domestic Wastewater

- When dilution water is not seeded, the BOD value is expressed in mg/L according to the following equation

$$BOD\ (\text{mg/L}) = \frac{D_1 - D_5}{P}$$

Composition of Domestic Wastewater

- The relationship between BOD at any time and ultimate carbonaceous BOD is given by the following equation (Hammer, 1986):

$$BOD_t = L(1 - 10^{-kt})$$

Composition of Domestic Wastewater

- where L= ultimate BOD (mg/L); k = BOD rate constant with a value of approximately 0.1/day for domestic wastewaters;

Composition of Domestic Wastewater

ii. Chemical Oxygen Demand

Chemical oxygen demand (COD) is the amount of oxygen necessary to oxidize the organic carbon completely to CO2, H2O, and ammonia (Sawyer and McCarty, 1967

Composition of Domestic Wastewater

iii. Total Organic Carbon

- expressing organic matter is in terms of its carbon content.

- Carbon is the primary constituent of organic matter, and hence the chemical formula of every organic compound will reflect the extent of carbon present in that compound.

-

Composition of Domestic Wastewater

- Known concentrations of such chemical compounds in a given wastewater will thus enable us to theoretically calculate the carbon present in that wastewater per liter of solution.

Overview of Wastewater Treatment

- Physical forces as well as chemical and biological processes drive the treatment of wastewater.

- Treatment methods that rely on physical forces are called unit operations.

Overview of Wastewater Treatment

- These include screening, sedimentation, filtration, or flotation.
- Treatment methods based on chemical and biological processes are called unit processes.

OVERVIEW OF WASTEWATER TREATMENT

- Chemical unit processes include disinfection, adsorption, or precipitation.

- Biological unit processes involve microbial activity, which is responsible for organic matter degradation and removal of nutrients.

(Metcalf and Eddy, 1991).

Overview of Wastewater Treatment

Wastewater treatment comprises the following four steps

1. Preliminary treatment. The objective of this operation is to remove debris and coarse materials that may clog equipment in the plant.

2. Primary treatment. Treatment is brought about by physical processes (unit operations) such as screening and sedimentation.

Overview of Wastewater Treatment

3. Secondary treatment. Biological (e.g., activated sludge, trickling filter, oxidation ponds) and chemical (e.g., disinfection) unit processes are used to treat wastewater.

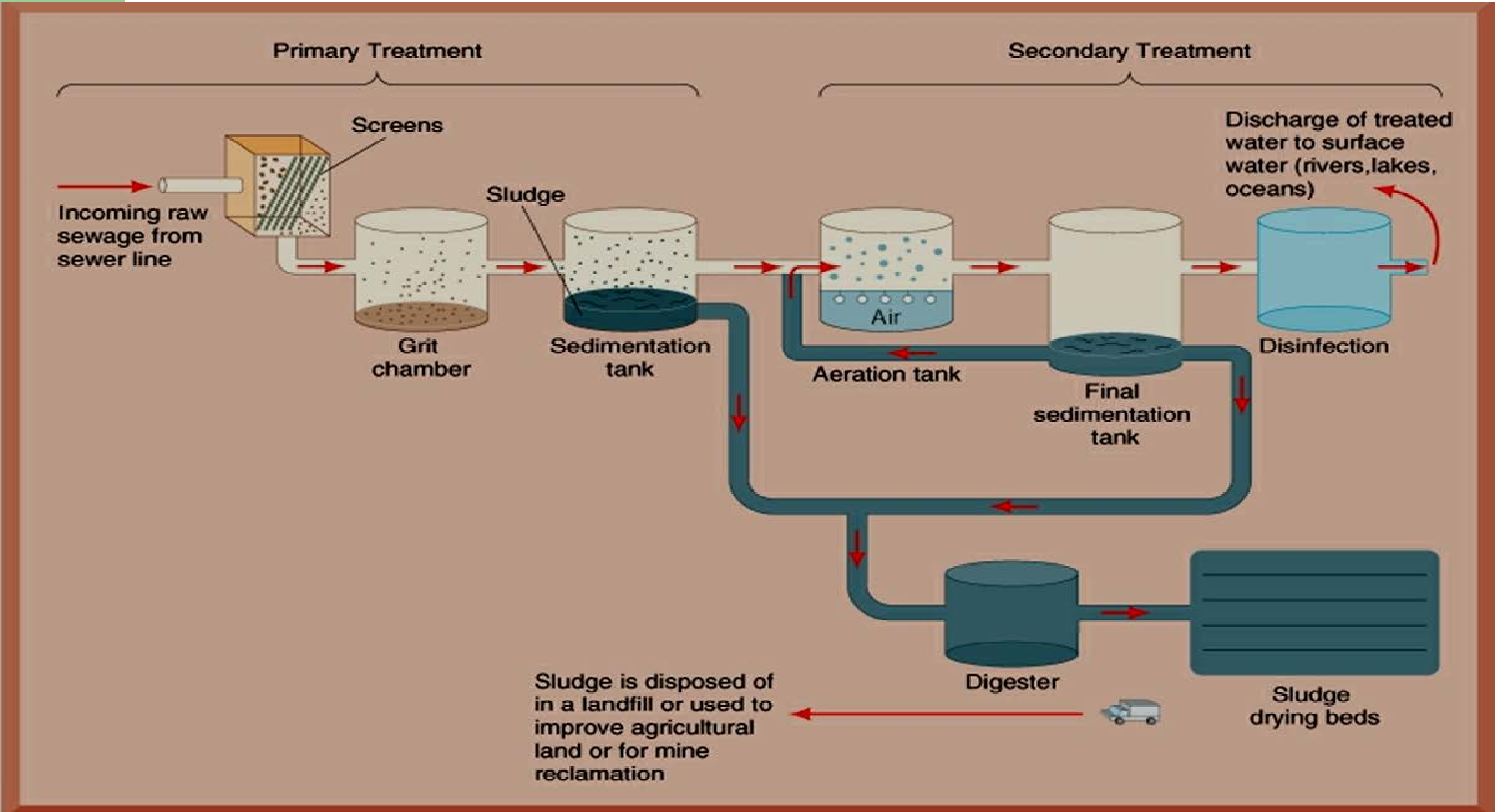
Nutrient removal also generally occurs during secondary treatment of wastewater.

Overview of Wastewater Treatment

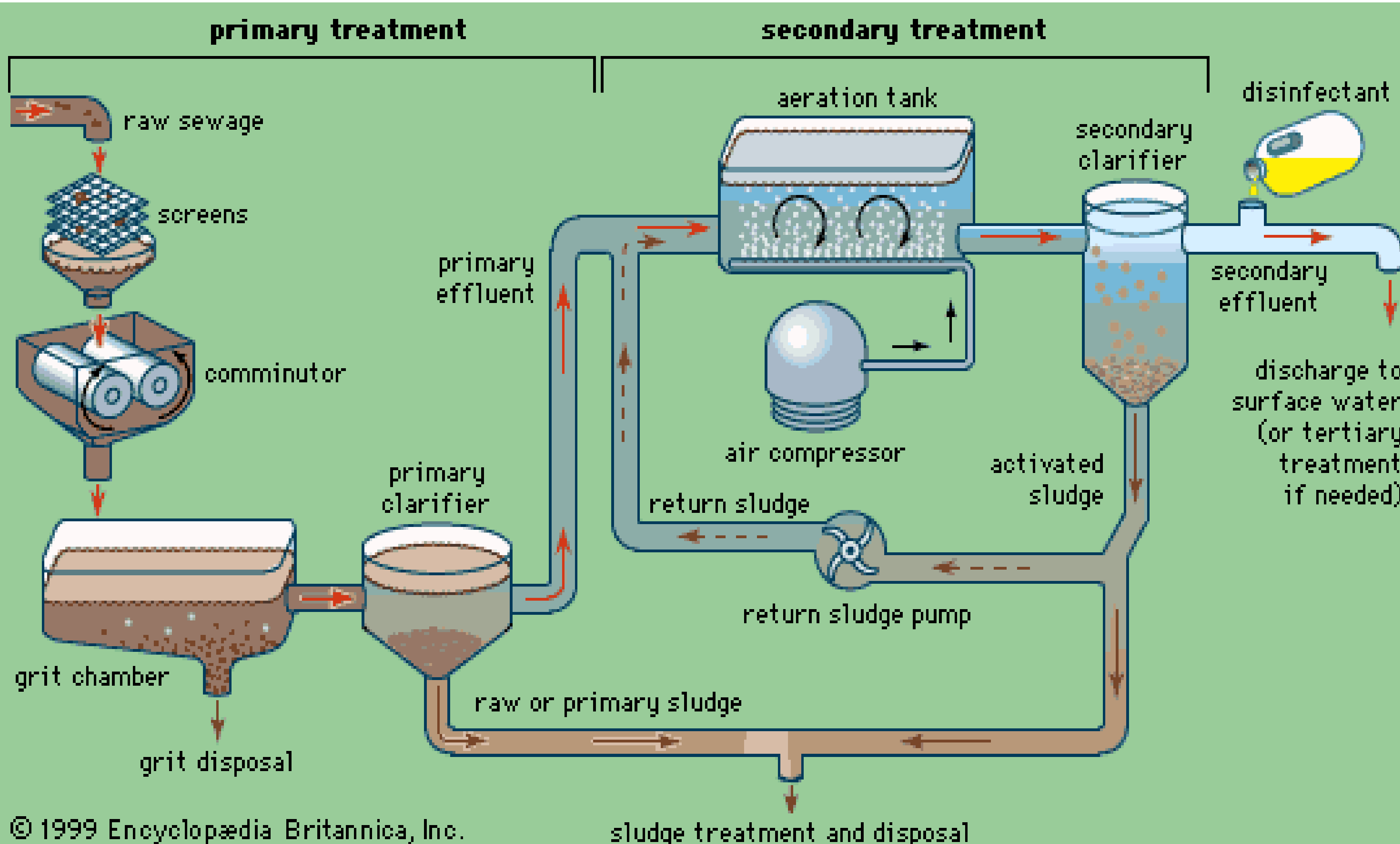
4. Tertiary or advanced treatment. Unit operations and chemical unit processes are

used to further remove BOD, nutrients, pathogens, and parasites, and sometimes toxic substances.

Overview of Wastewater Treatment



Overview of Wastewater Treatment



Trickling Filter

$$BOD\ (\text{mg/L}) = \frac{D_1 - D_5}{P}$$

Exercise:

$$BOD_t = L(1 - 10^{-kt})$$

- BOD of a wastewater sample: 5 mL of wastewater is added to a 300 mL BOD bottle. Add dilution water to the bottle until obtaining a volume of 300 mL. The initial DO concentration was 7.8 mg/L and the final DO concentration was 4.3 mg/L after five-day incubation.

(a) Calculate the BOD5 of the sample.

(b) Calculate the ultimate BOD (assuming a k value of 0.1).

THE END