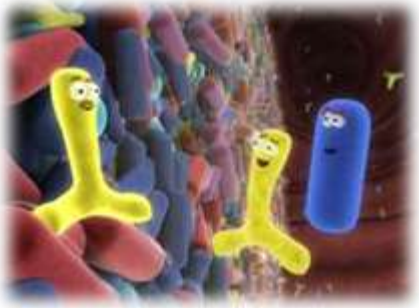
WASTEWATER MICROBIOLOGY



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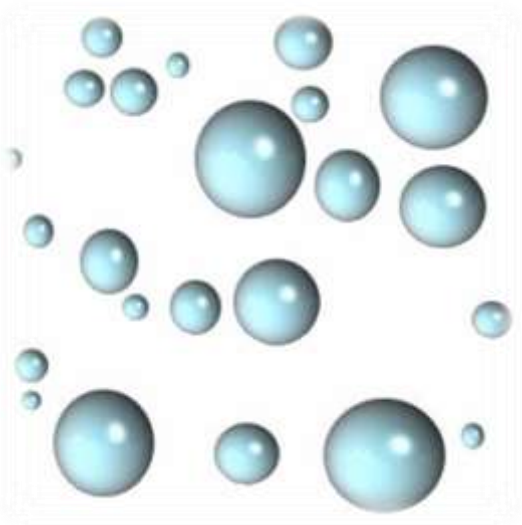
Wastewater

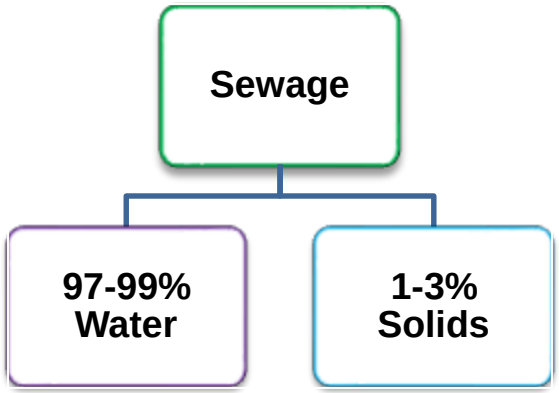
- ✓ Water that has been used by people and is disposed into a receiving water body with altered physical and/or chemical parameters is defined as **wastewater**.

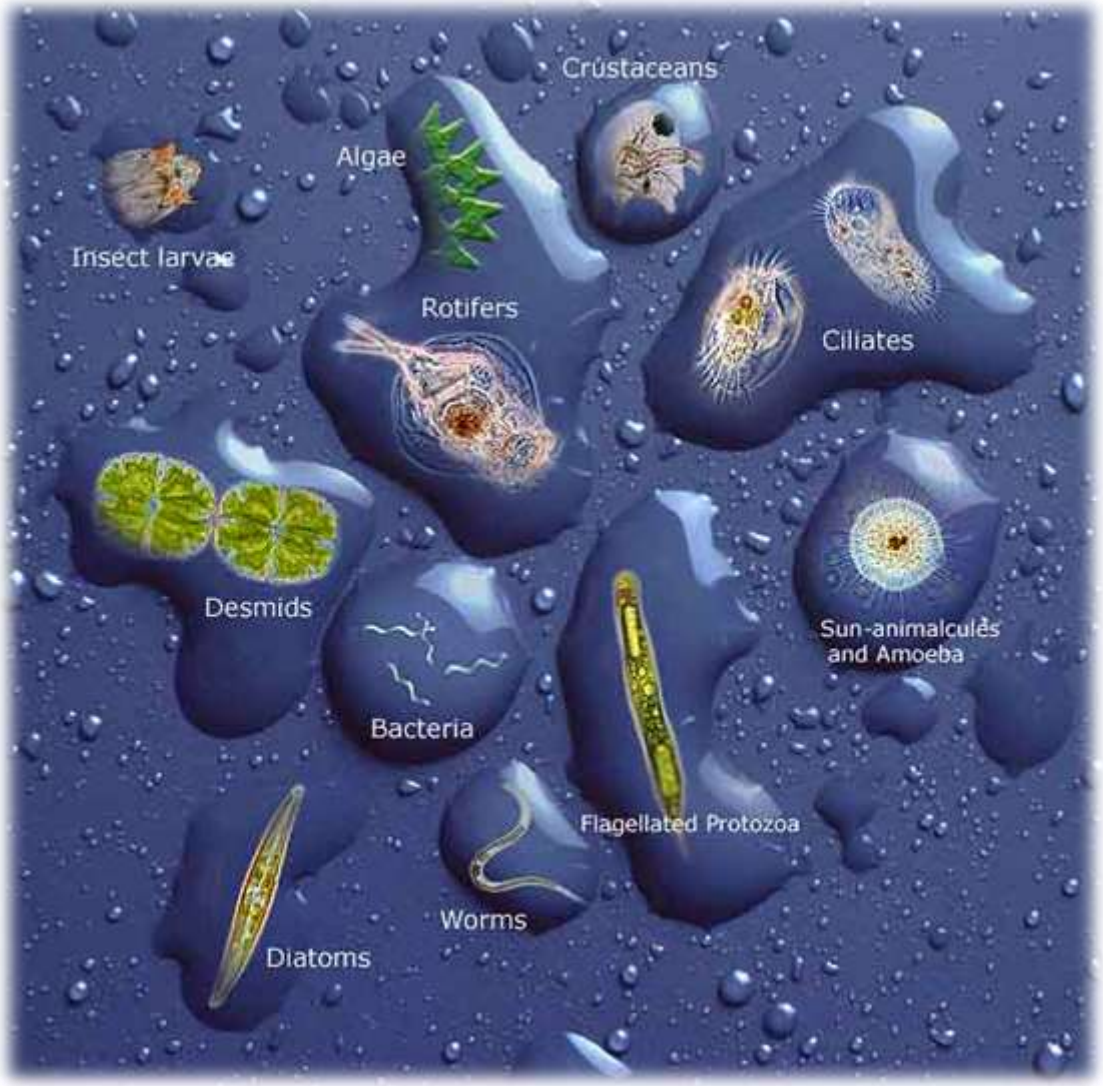
- ✓ If only the physical parameters of the water were changed, e.g., resulting in an elevated temperature after use as a coolant, treatment before final disposal into a surface water may require only cooling close to its initial temperature.

- ✓ If the water, is contaminated with soluble or insoluble organic or inorganic material, a combination of mechanical, chemical, and/or biological purification procedures may be required.

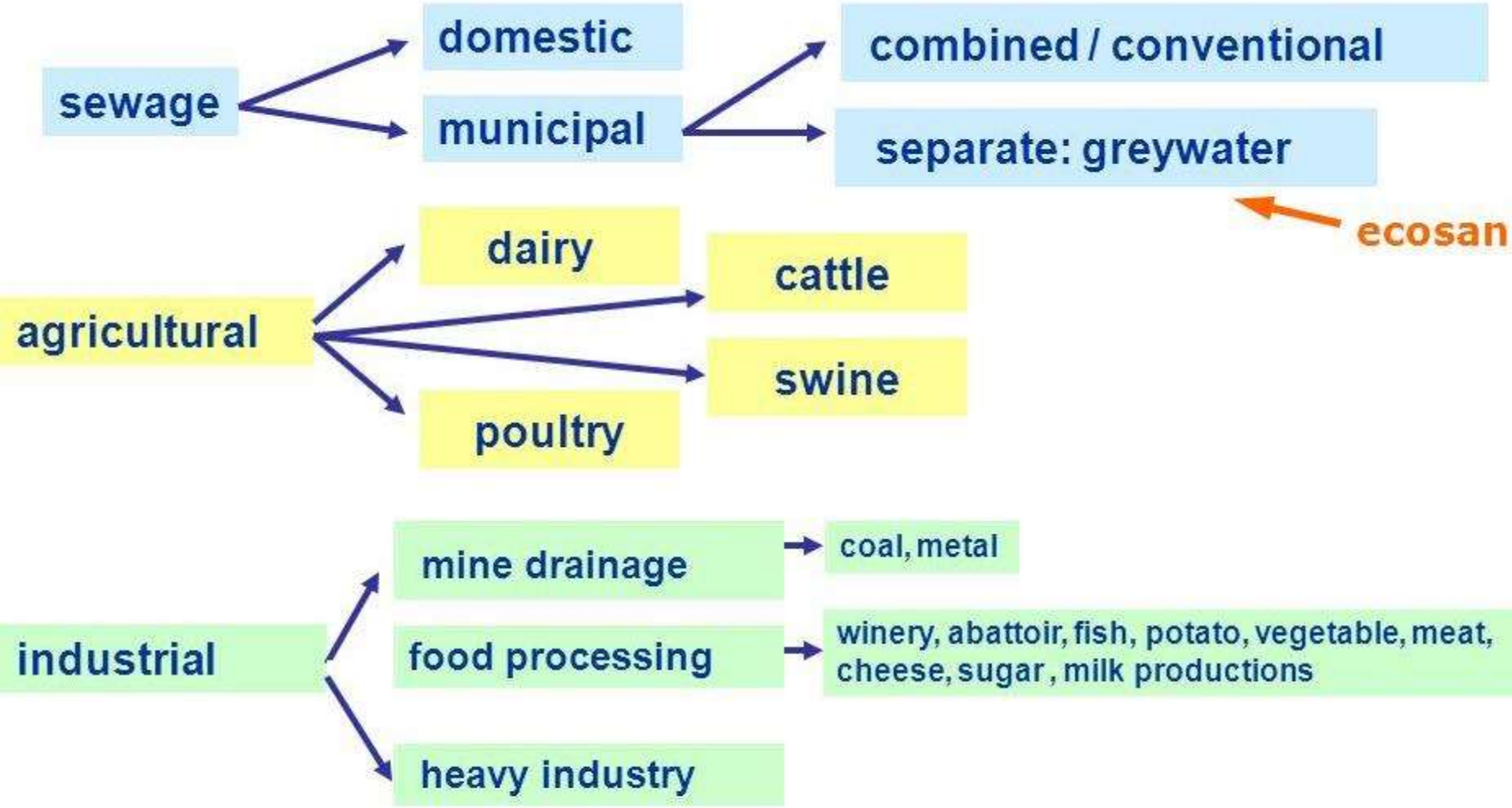


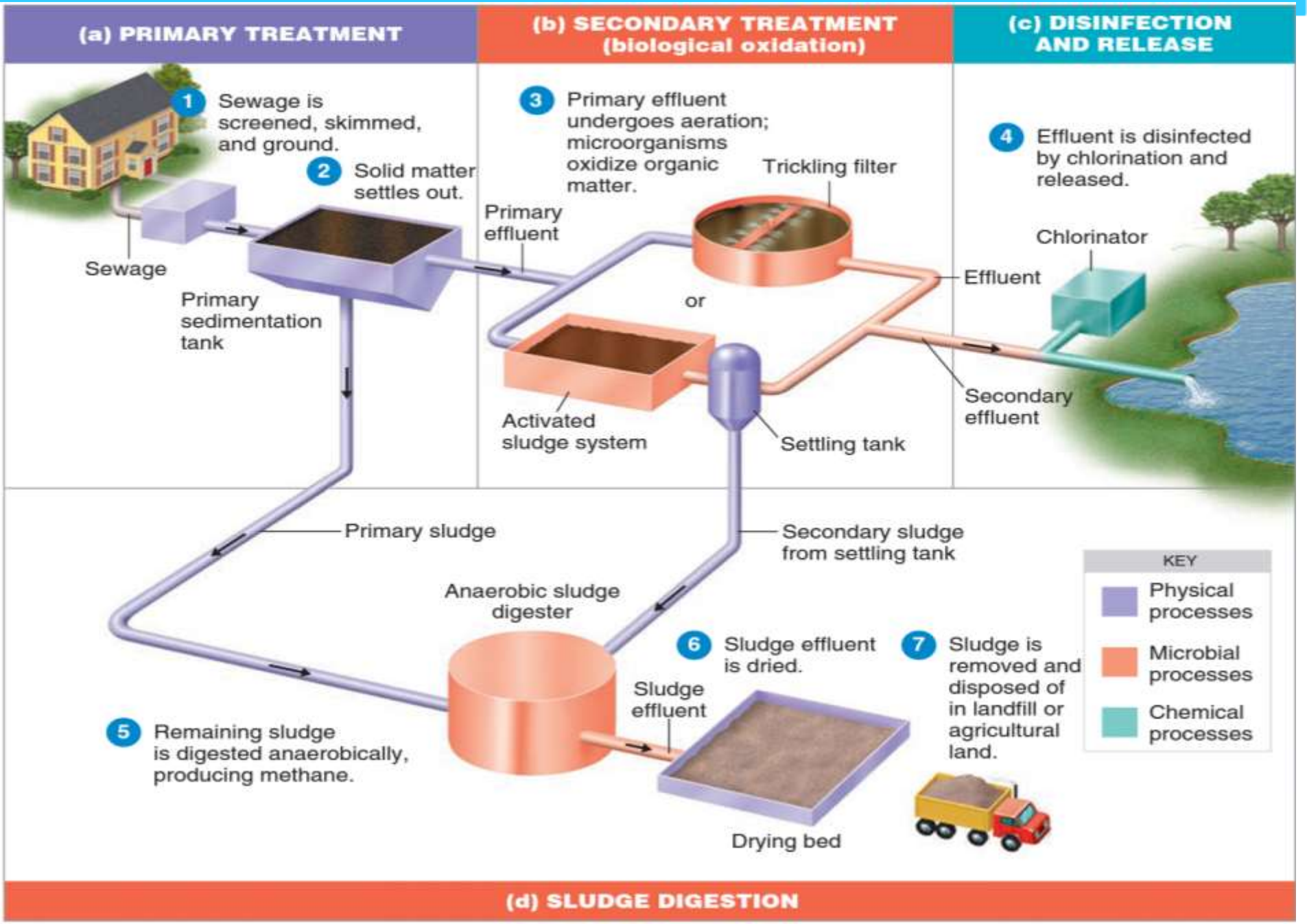
The composition of sewage mainly depends upon source from which it comes and varies from place to place and season to season.

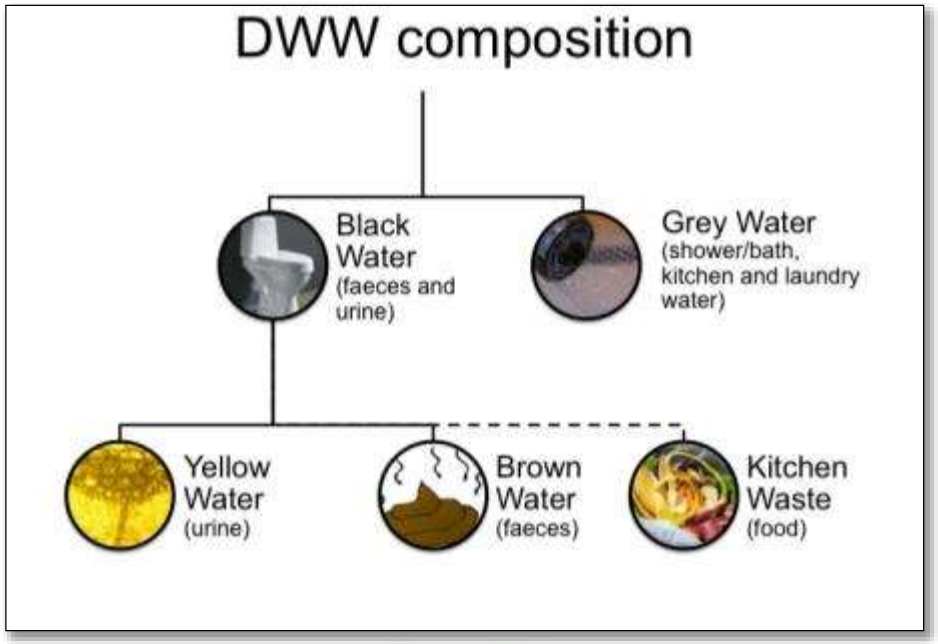




- **Biowaste of animal origin such as that contained in sewage and soiled animal bedding contains unabsorbed fats, proteins and carbohydrates, resulting from incomplete digestion of ingested food of animal and plant origin.**
- **In addition, abattoir waste would include all of the above and a substantial proportion of fats and protein, derived from the slaughtered animal. In addition, materials excreted by the animal include metabolic breakdown products such as urea and other small nitrogen-containing materials, for example partially degraded bile pigments.**
- **Live and dead bacteria, normally resident in animal gut are also present in the biowaste and so contribute their own fats, proteins, carbohydrates and nucleic acids. In addition to all the components listed above, biowaste of plant origin will contain cellulose, hemicelluloses and lignin.**
- **Cellulose is worthy of note given that estimates of over 50% of the total organic carbon in this biosphere is to be found in the form of cellulose. This is unsurprising, since wood is approximately 50% cellulose and cotton is almost 100% cellulose.**


sewage
domestic
municipal
combined / conventional
separate: greywater
ecosan
agricultural
dairy
cattle
swine
poultry
industrial
mine drainage
coal, metal
food processing
winery, abattoir, fish, potato, vegetable, meat, cheese, sugar , milk productions
heavy industry
runoff
urban
highway
airport
field crop
nursery
greenhouse


(a) PRIMARY TREATMENT
(b) SECONDARY TREATMENT (biological oxidation)
(c) DISINFECTION AND RELEASE
1 Sewage is screened, skimmed, and ground.
2 Solid matter settles out.
Sewage
Primary sedimentation tank
3 Primary effluent undergoes aeration; microorganisms oxidize organic matter.
Primary effluent
Trickling filter
or
Activated sludge system
Settling tank
4 Effluent is disinfected by chlorination and released.
Chlorinator
Effluent
Secondary effluent
Primary sludge
Secondary sludge from settling tank
Anaerobic sludge digester
5 Remaining sludge is digested anaerobically, producing methane.
6 Sludge effluent is dried.
Sludge effluent
Drying bed
7 Sludge is removed and disposed of in landfill or agricultural land.
KEY
Physical processes
Microbial processes
Chemical processes
(d) SLUDGE DIGESTION

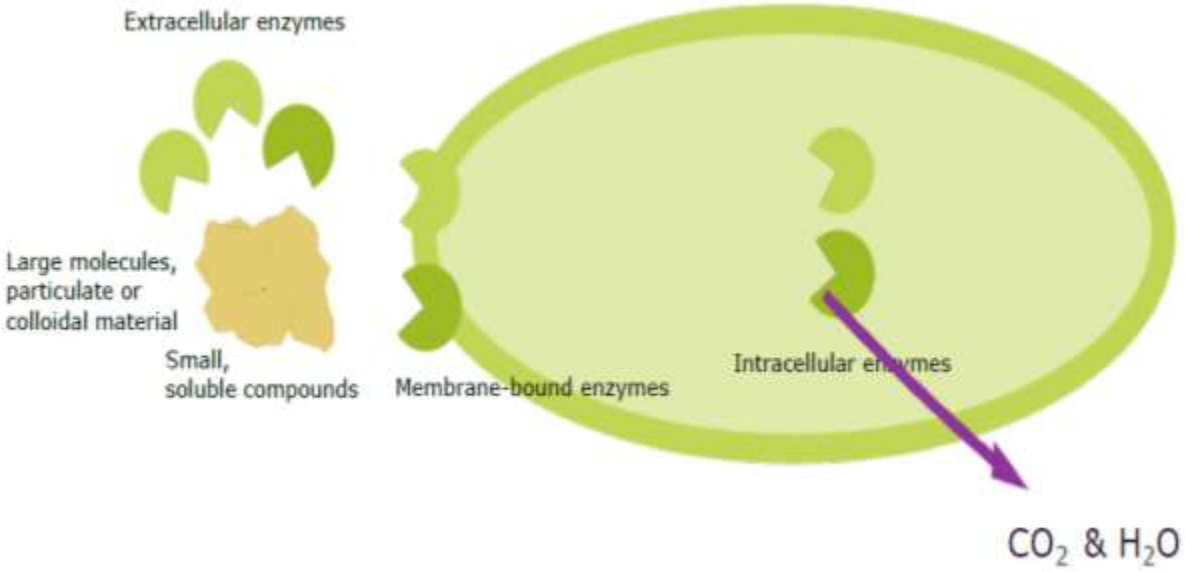


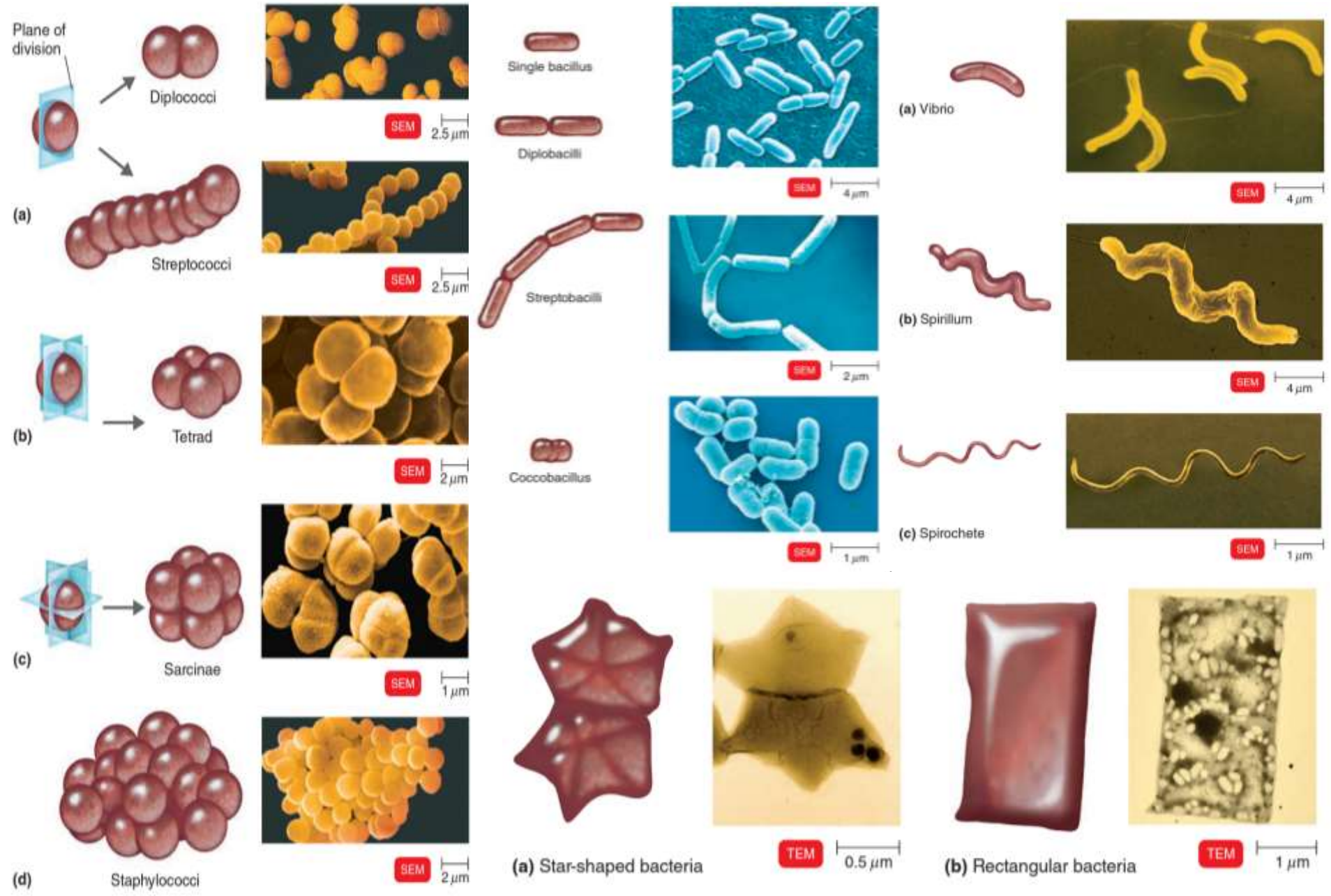
Microorganisms are Ubiquitous

We are growing bacteria to clean the water…

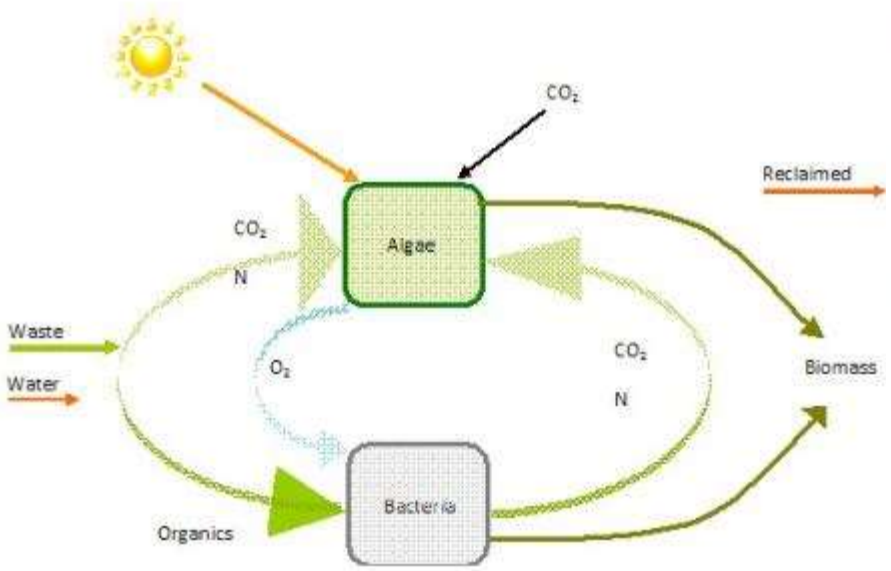
Table 3.2 Constituents present in domestic wastewater (based on Henze *et al.*, 2001)

Wastewater constituents		
Microorganisms	Pathogenic bacteria, virus and worms eggs	Risk when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers, lakes and fjords	Fish death, odours
Other organic materials	Detergents, pesticides, fat, oil and grease, colouring, solvents, phenols, cyanide	Toxic effect, aesthetic inconveniences, bio accumulation in the food chain
Nutrients	Nitrogen, phosphorus, ammonium	Eutrophication, oxygen depletion, toxic effect
Metals	Hg, Pb, Cd, Cr, Cu, Ni	Toxic effect, bioaccumulation
Other inorganic materials	Acids, for example hydrogen sulphide, bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora and fauna
Odour (and taste)	Hydrogen sulphide	Aesthetic inconveniences, toxic effect
Radioactivity		Toxic effect, accumulation




Plane of division
Diplococci
(a)
Streptococci
(b)
Tetrad
(c)
Sarcinae
(d)
Staphylococci
SEM
2.5 μm
SEM
2.5 μm
SEM
2 μm
SEM
1 μm
SEM
2 μm
Single bacillus
Diplobacilli
Streptobacilli
Coccobacillus
SEM
4 μm
SEM
2 μm
SEM
1 μm
(a) Vibrio
(b) Spirillum
(c) Spirochete
SEM
4 μm
SEM
4 μm
SEM
1 μm
(a) Star-shaped bacteria
TEM
0.5 μm
(b) Rectangular bacteria
TEM
1 μm

- ✓ The stabilization of wastewater is accomplished biologically using a variety of microorganisms.
- ✓ The microorganisms convert colloidal and dissolved carbonaceous organic matter into various gases and into protoplast.



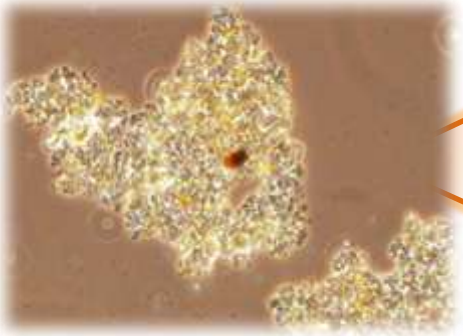
A wastewater treatment plant is basically a bug factory.

Organism	Description
Bacteria	• Unicellular organisms • Present in various forms and sizes • Main organisms responsible for the stabilisation of organic matter • Some bacteria are pathogenic, causing mainly intestinal diseases
Archaea	• Similar to bacteria in size and basic cell components • Different from bacteria in their cell wall, cell material and RNA composition • Important in anaerobic processes
Algae	• Autotrophic photosynthetic organisms, containing chlorophyll • Important in the production of oxygen in water bodies and in some sewage treatment processes • In lakes and reservoirs they can proliferate in excess, deteriorating the water quality
Fungi	• Predominantly aerobic, multicellular, non-photosynthetic, heterotrophic organisms • Also of importance in the decomposition of organic matter • Can grow under low pH conditions
Protozoa	• Usually unicellular organisms without cell wall • Majority is aerobic or facultative • Feed themselves on bacteria, algae and other microorganisms • Essential in biological treatment to maintain an equilibrium between the various groups • Some are pathogenic
Viruses	• Parasitic organisms, formed by the association of genetic material (DNA or RNA) and a protein structure • Pathogenic and frequently difficult to remove in water or wastewater treatment
Helminths	• Higher-order animals • Helminth eggs present in sewage can cause illnesses

Most organisms in biological wastewater treatment plants-

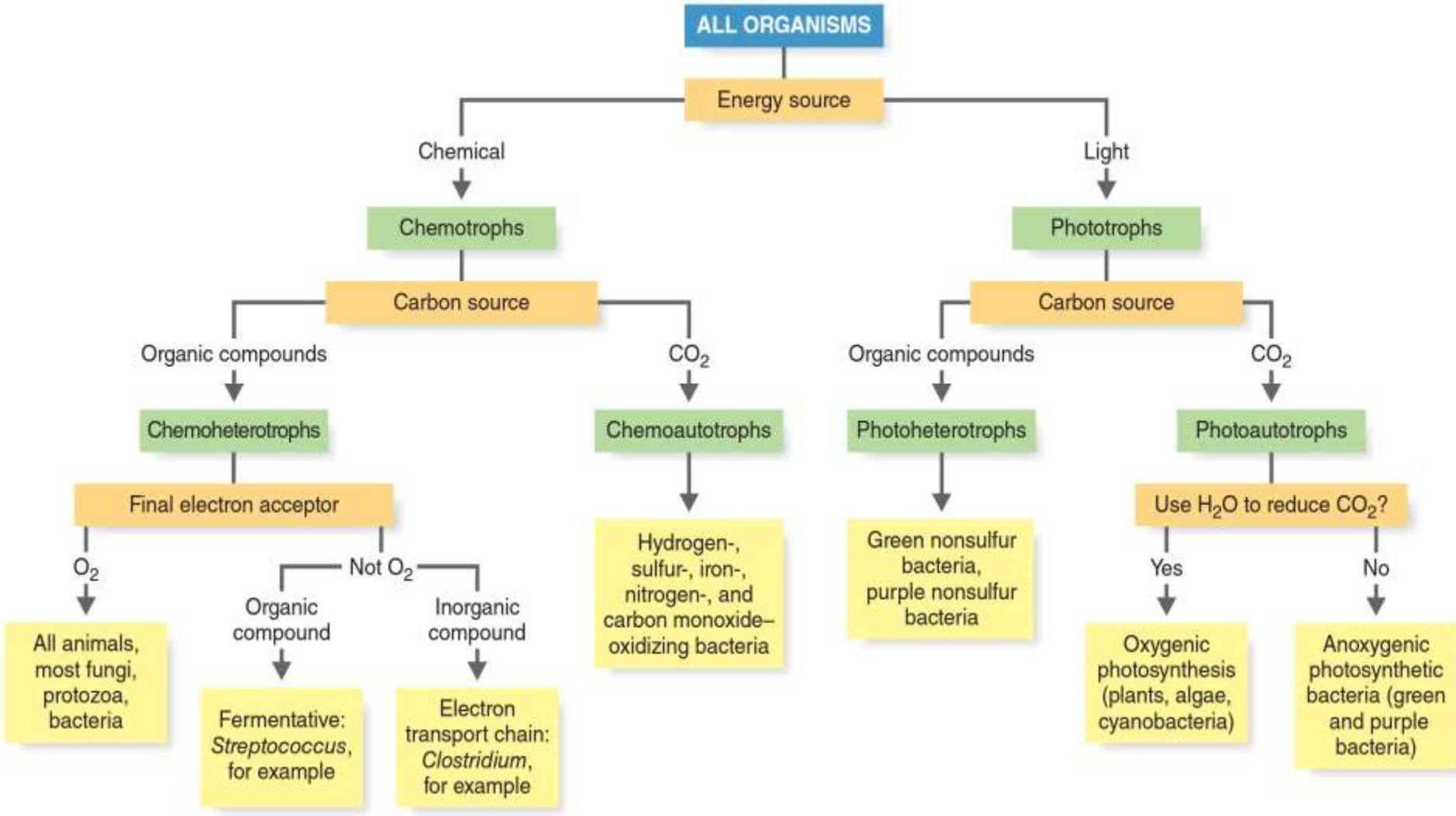
- Microscopic
- Macroscopic

In size



400x

7200x

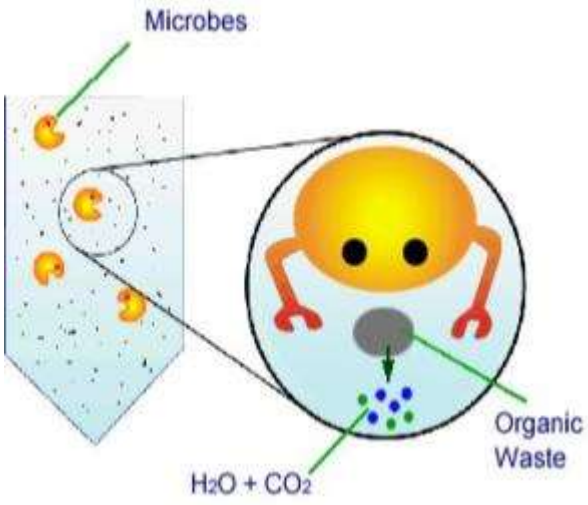

ALL ORGANISMS
Energy source
Chemical
Light
Chemotrophs
Phototrophs
Carbon source
Carbon source
Organic compounds
CO_2
Organic compounds
CO_2
Chemoheterotrophs
Chemoautotrophs
Photoheterotrophs
Photoautotrophs
Final electron acceptor
Use H_2O to reduce CO_2?
O_2
Not O_2
Yes
No
Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide–oxidizing bacteria
Green nonsulfur bacteria, purple nonsulfur bacteria
Organic compound
Inorganic compound
All animals, most fungi, protozoa, bacteria
Fermentative: *Streptococcus*, for example
Electron transport chain: *Clostridium*, for example
Oxygenic photosynthesis (plants, algae, cyanobacteria)
Anoxygenic photosynthetic bacteria (green and purple bacteria)

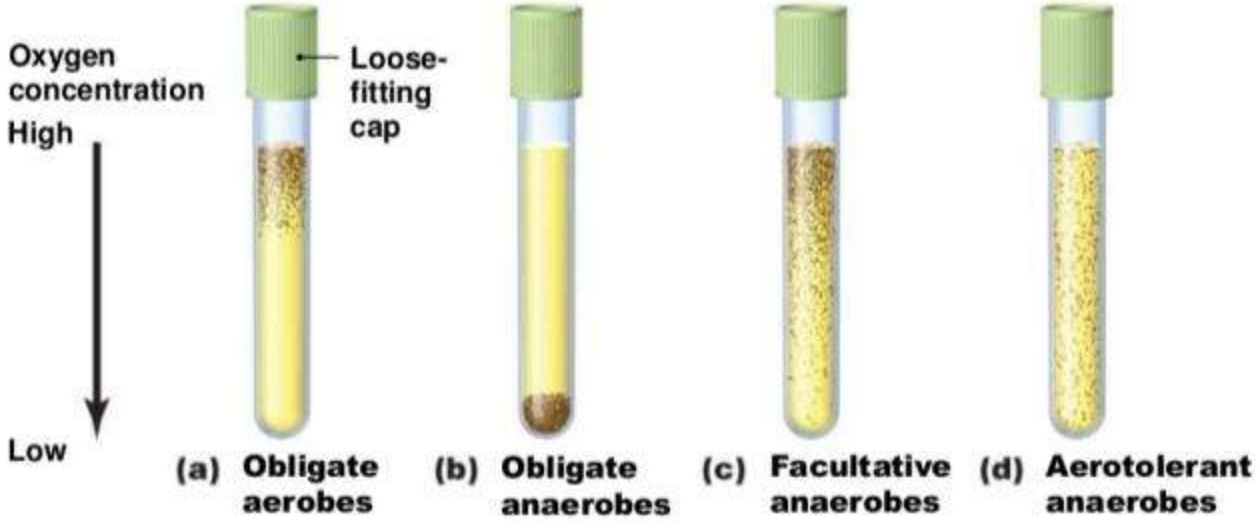
Bacteria in Wastewater Treatment Systems

- ❖ Greatest numerical importance !!!!!
- ✓ **Vital role** in conversion of organic matter present to less complex compounds.
- ❖ Size- 0.2- 2.0 µm in diameter
- ✓ Large surface area in relation to their body volume and their associated high metabolic and reproductive rates.
- ✓ Not all are harmful,-but, a number of them cause **water-related diseases** in human and animals. eg; cholera, dysentery, typhoid fever, salmonellosis and gastroenteritis
- ❖ **Heterotrophic** bacteria predominate (obtain their energy from the carbonaceous organic matter in wastewater effluent- biomass)
- ✓ Found entangled in flocs (activated sludge)-important role in biological treatment
- ❑ Filamentous bacteria can cause serious problem in settling and foaming **!!!!**

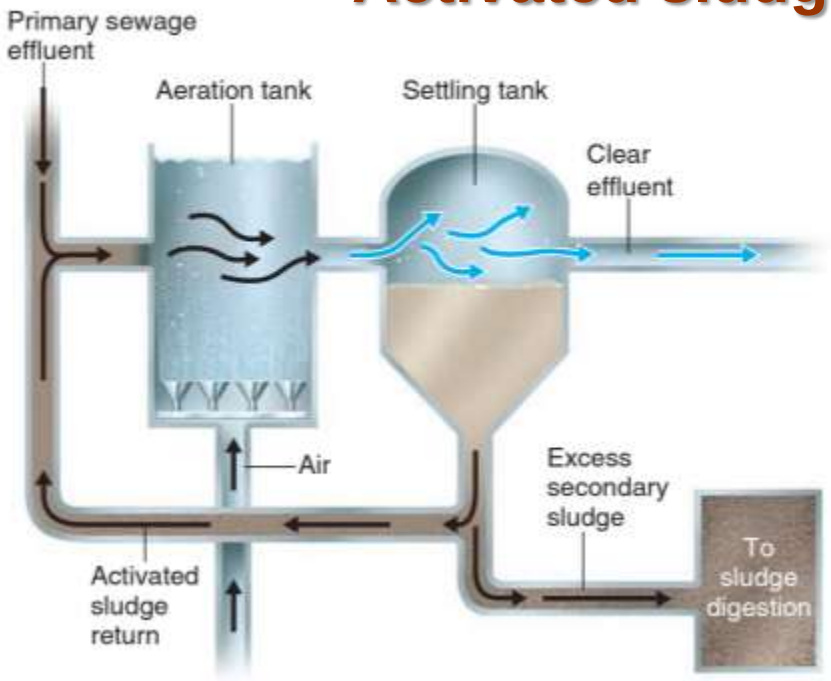
- ✓ Some important bacteria genera that are found in WW treatment systems are *Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas, Zooglea* and *Acinetobacter*
- ✓ Some may be chemolithotrophic and oxidize NH_3 (Nitrosomonas), NO_2^- (Nitrobacter), and H_2S (Thiothrix).
- ✓ Filamentous bacteria such as *Sphaerotilus* may be found to cause bulked sludges

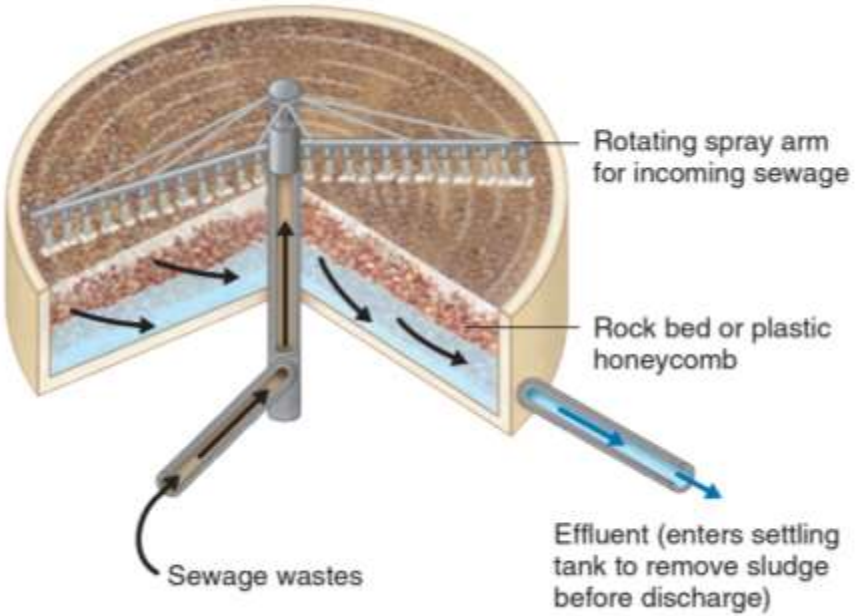
Microbes oxidize organic waste to CO_2 & H_2O and produce flocculent settleable solids

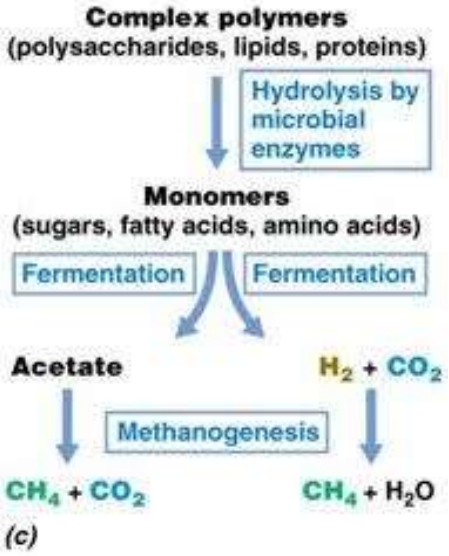




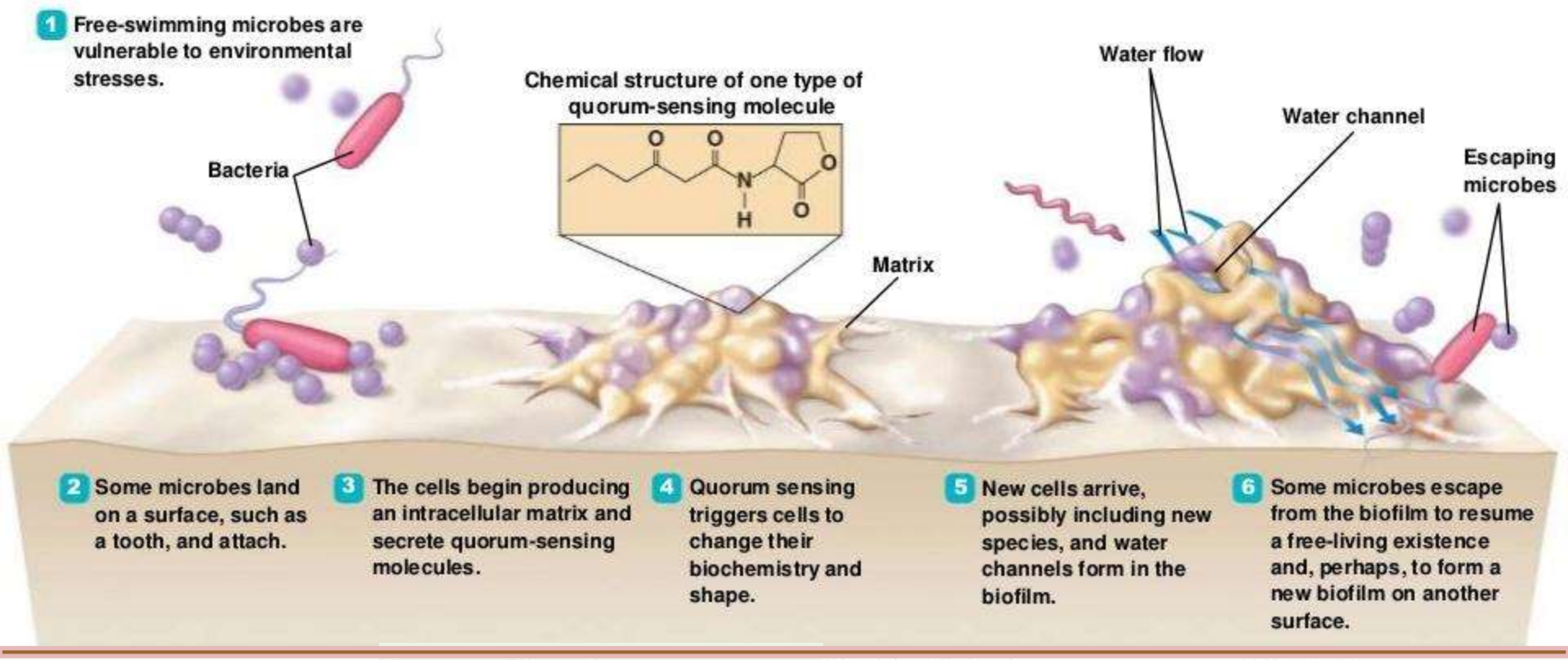
Activated sludge and Trickling filter system



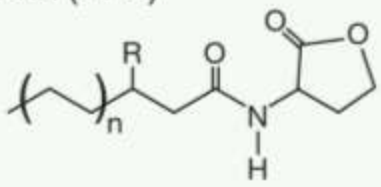




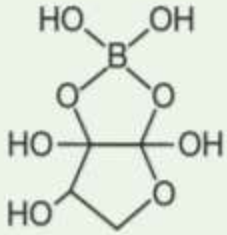
Biological treatment of Wastewater



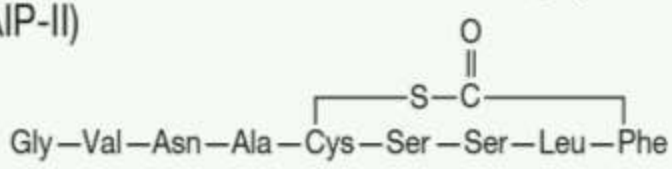
N-acyl homoserine lactone (AHL)



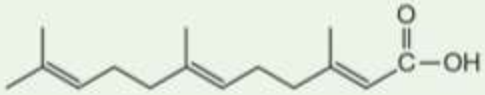
Furanosylborate (AI-2)



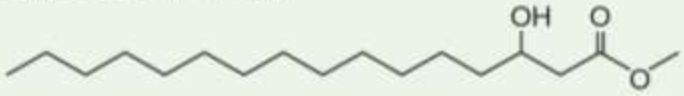
Cyclic thiolactone (AIP-II) — *Staphylococcus aureus*



Farnesoic acid — *Candida albicans*

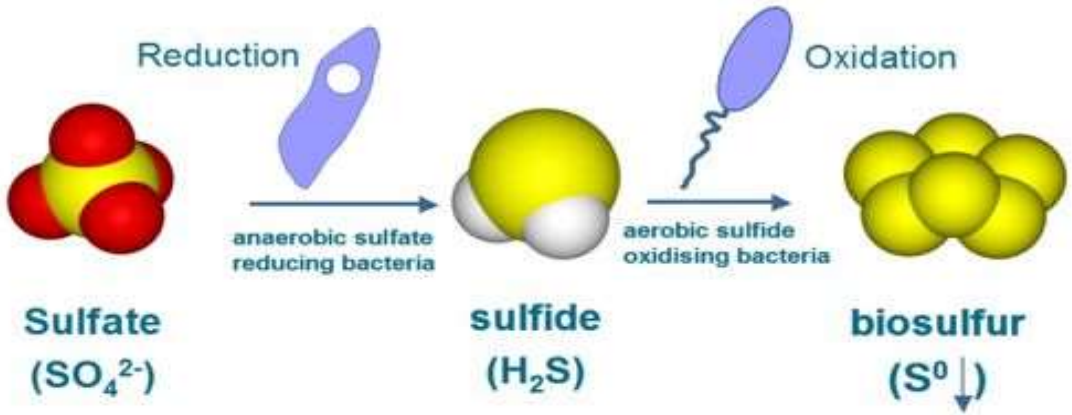


Hydroxy-palmitic acid methyl ester (PAME) — *Ralstonia solanacearum*



Some important bacterial signaling molecules……

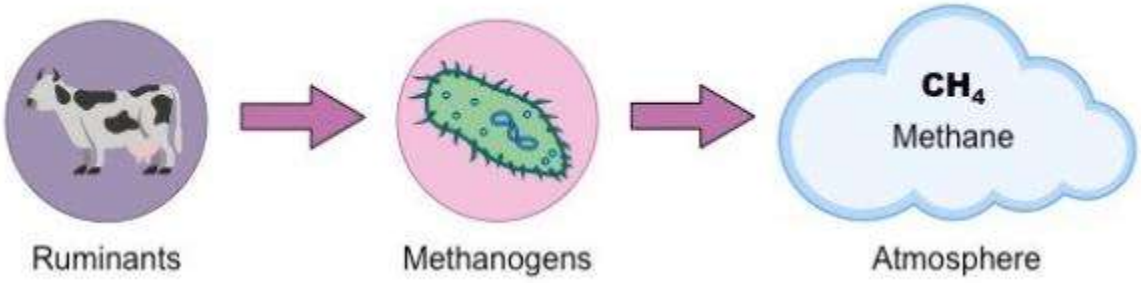
- ✓ *Zooglea ramigera* and similar bacteria -slime matrix generators- colonised by heterotrophic microbes eg; *Beggiatoa alba*, *Sphaerotilus mutans*, *Achromobacter*, *Pseudomonas*, *Flavobacterium etc.*
- ✓ *Zooglea* are exopolysaccharide- producing bacteria that produce typical finger-like projections and are found in wastewater and other organically enriched environments.
- ✓ These finger-like projections consist of aggregates of *Zooglea* cells surrounded by a Polysaccharide matrix



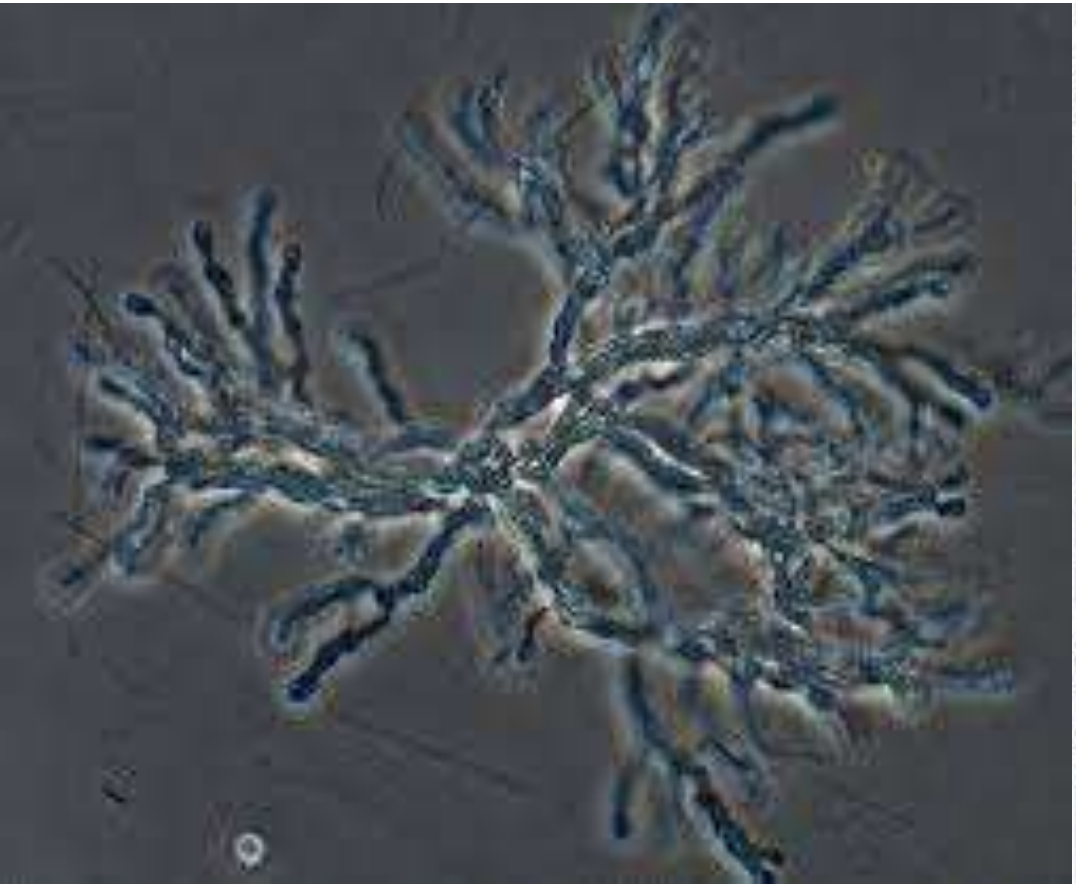
Most of the sulphide produced from sewage is from bacteria that reduce sulphates, such as *Desulfovibrio desulfuricans*

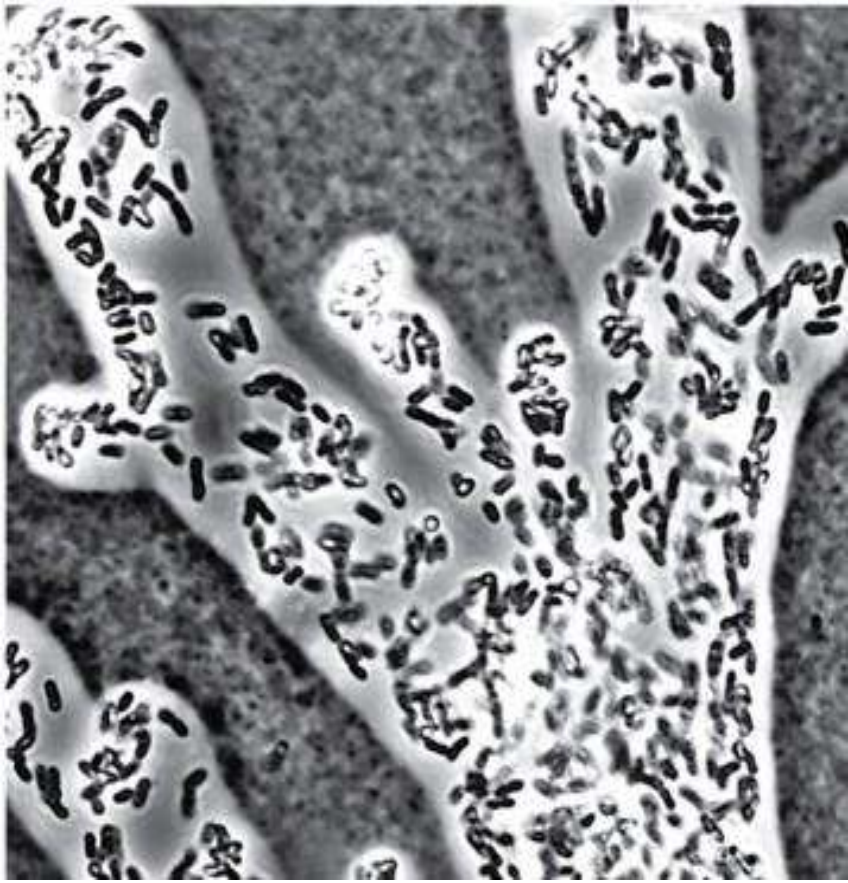
Carbon Source: Methane

Methanobacterium– responsible for methane production



Zooglea ramigera





- ✓ Activated sludge flocs also harbor autotrophic bacteria such as nitrifiers (*Nitrosomonas, Nitrobacter*), which **convert NH_4^+ to NO_3^-**, phototrophic bacteria such as the **purple nonsulfur bacteria**
- ✓ Floc particles -clusters of bacteria that break down waste.
- ✓ Filamentous bacteria form, **trichomes** or filaments -provide a backbone for the floc particles, allowing particle growth and withstand the shearing action of various treatment process.
- ✓ Excessive numbers or length, -cause solid**/**liquid separation or settleability problems
- ✓ Major genera in the flocs are *Zooglea*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Achromobacter*, *Corynebacterium*, *Brevibacterium*, *Acinetobacter*, *Bacillus spp*., as well as filamentous microorganisms.
- ✓ Some examples of filamentous microorganisms are the sheathed bacteria (e.g., *Sphaerotilus*) and gliding bacteria (e.g., *Beggiatoa*, *Vitreoscilla*), which are responsible for **sludge bulking**.
- ✓ The floc particles, serve as sites on which waste can be absorbed and broken down.

Pathogenic Bacteria

- ✓ Most common microbial pollutants in wastewater.
- ✓ The presence of pathogenic bacteria -indicated using tests for total and faecal coliforms
- ✓ **Coliforms-** indicators of faecal contamination.
- ✓ ***Escherichia coli*** -good and reliable **indicator for faecal pollution** from animal and human sources since, it is known not to last for long periods outside the faecal environment

The tests for total and faecal coliforms can be carried out, using

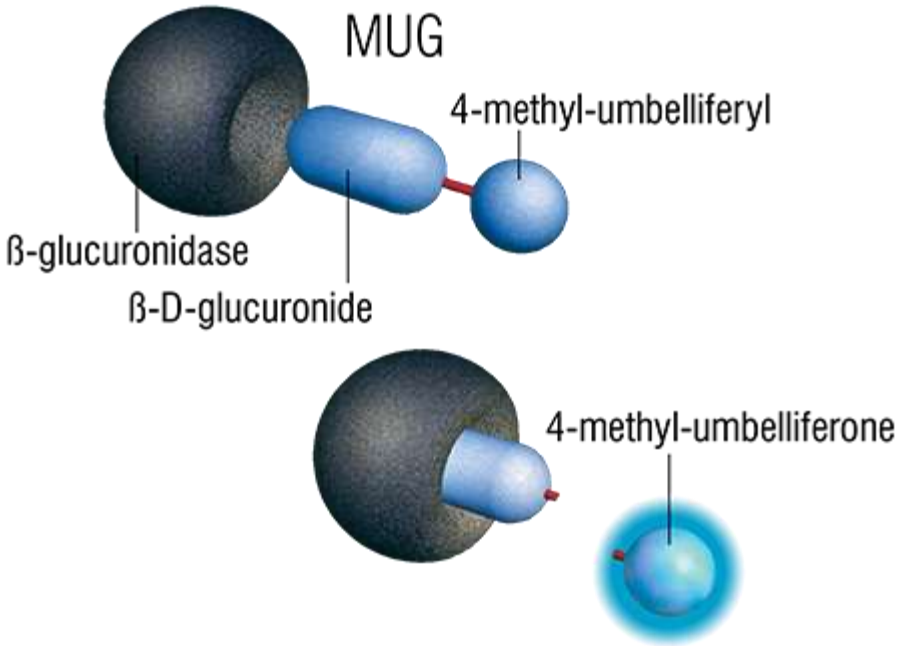
a. **Traditional** and/ or
b. **Enzymatic** methods.

- ✓ Traditionally -**multiple-tube fermentation** (MPN) or by **MFT**
- ✓ A newer and more convenient method of detecting coliforms, specifically the fecal coliform ***E.coli***, makes use of media containing substrates such as

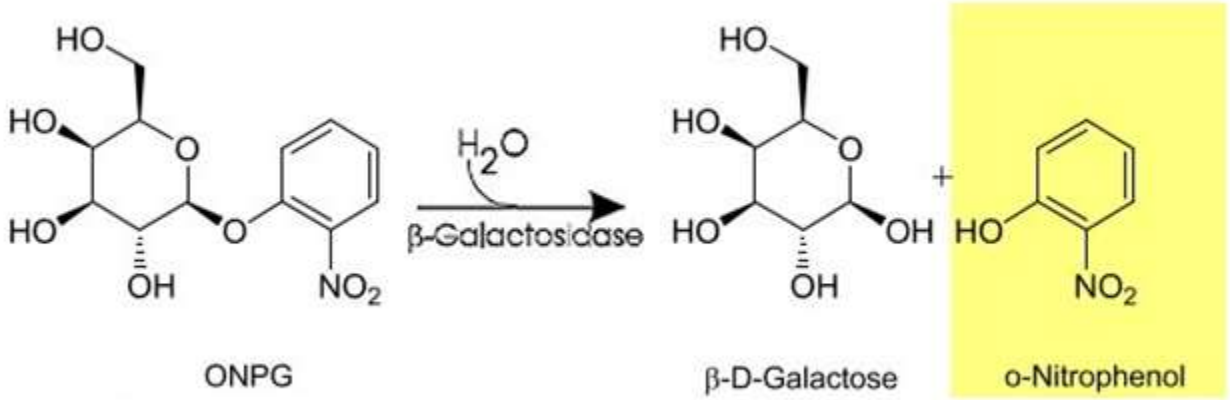
- **o-nitrophenyl-β-D-galactopyranoside (ONPG)** and
- **4-methylumbelliferyl-β-D-glucuronide (MUG).**

- These simple tests, can detect the presence or absence of coliforms or ***E. coli*** and can be combined with the multiple-tube method to enumerate them.

E. coli

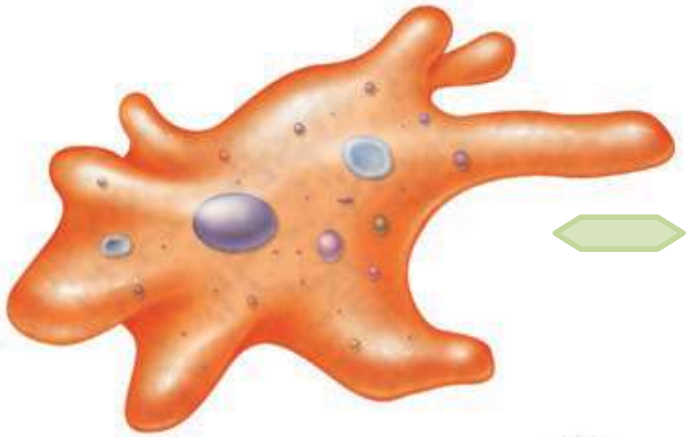

MUG
4-methyl-umbelliferyl
ß-glucuronidase
ß-D-glucuronide
4-methyl-umbelliferone

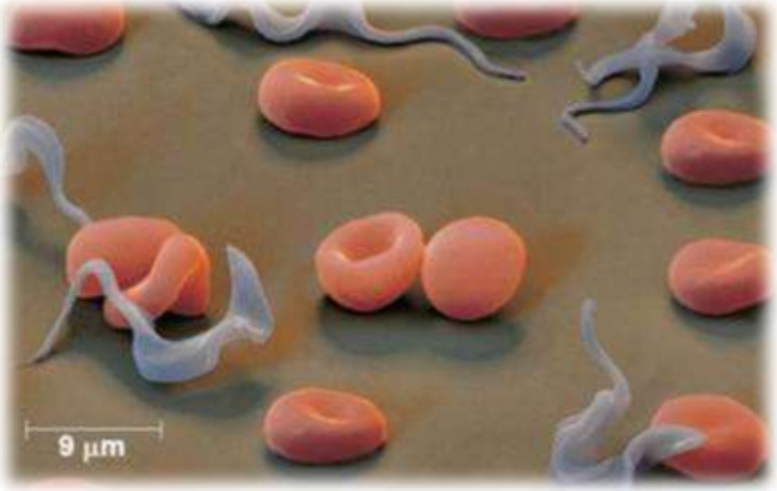

Uncontam-
inated
sample
Coliforms
Fecal
coliforms

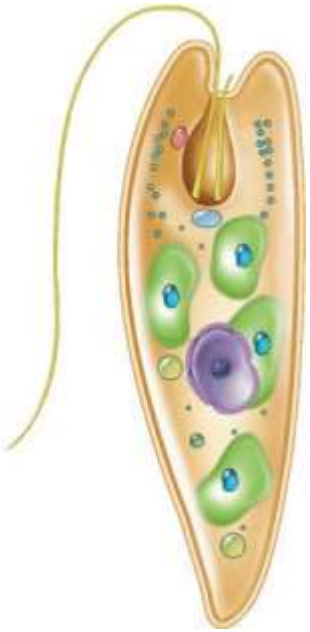

HO
HO
HO
O
OH
O
NO2
H2O
β-Galactosidase
HO
HO
HO
O
OH
OH
+
HO
NO2
ONPG
β-D-Galactose
o-Nitrophenol

Protozoa

Motile, Eukaryotic Unicellular Protist



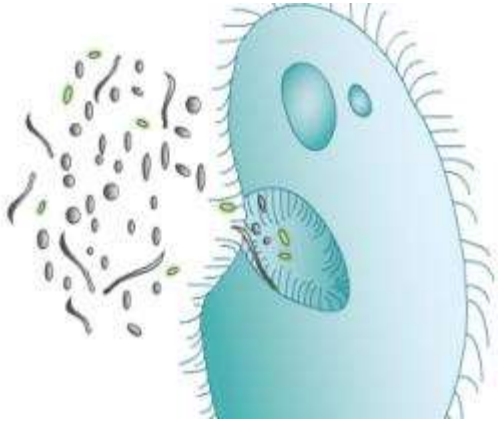




- **The term *protozoa means "first animals,"***
- **Protozoa reproduce asexually by fission, budding, or schizogony.**
- **Schizogony is multiple fission; the nucleus undergoes multiple divisions before the cell divides.**
- **After many nuclei are formed, a small portion of cytoplasm concentrates around each nucleus, and then the single cell separates into daughter cells**
- **Protozoa are mostly aerobic heterotrophs, although many intestinal protozoa are capable of anaerobic growth.**
- **Two chlorophyll- containing groups, dinoflagellates and euglenoids, All protozoa live in areas with a large supply of water.**
- **Some protozoa transport food across the plasma membrane. However, some have a protective covering, or *pellicle, and thus* require specialized structures to take in food.**
- **Ciliates take in food by waving their cilia toward a mouthlike opening called a cytostome. Amebae engulf food by surrounding it with pseudopods and phagocytizing it.**
- **In all protozoa, digestion takes place in membrane-enclosed vacuoles, and waste may be eliminated through the plasma membrane or through a specialized anal pore.**

- ✓ Most are aerobic chemoheterotrophs,
- ✓ Advantage- prey on pathogenic bacteria- Hence, desirable in wastewater effluent.
- ✓ Excellent indicators of an aerobic environment & indicators of a **toxic environment** and are capable of exhibiting greater sensitivity to toxicity than bacteria.
- ✓ **An indication of possible toxicity in a treatment system is the absence of or a lack of mobility of protozoa.**
- ✓ They can be classified into five groups depending on their mode of locomotion,

1) **Free swimming ciliates,**
2) **Crawling ciliates,**
3) **Stalked and sessile ciliates,**
4) **Flagellates and**
5) **Amoeboid**



All of these three have short hair-like structures or cilia that beat in unison to produce water current for locomotion and capturing bacteria.

Ciliophora - contributes the greatest number of individuals to the microfauna.

Ciliates range from 500 to 10,000 individuals per ml of liquor

- ✓ The primary role of protozoa is to **clarify the effluent** through predation on (**Bacterivorous grazers**).
- ✓ Add weight to floc particles and improve their settleability.
- ✓ Produce and release secretions that coat and remove fine solids (colloids, dispersed cells, and particulate material) from the bulk solution to the surface of floc particles.
- ✓ Excrete growth-stimulating compounds that can enhance bacterial activity
- ✓ **Recycle nutrients** (nitrogen and phosphorus) through their excretions.
- ✓ Also cause flocculation resulting from secretion of a mucus like substance

Grazing on bacteria-remove senescent bacteria and maintain the population in a youthful physiological state **!!!**

Certain species - found in very large numbers

- *Vorticella*, *Opercularia*, *Carchesium* and swimmers like *Aspidisca* are dominant ones in Trickling filters.

- ***Opercularia microdiscum*** and ***Carchesium polypinum*** commonly present in RBC, where they colonize the biofilm.

Opercularia microdiscum

Vorticella convalaria

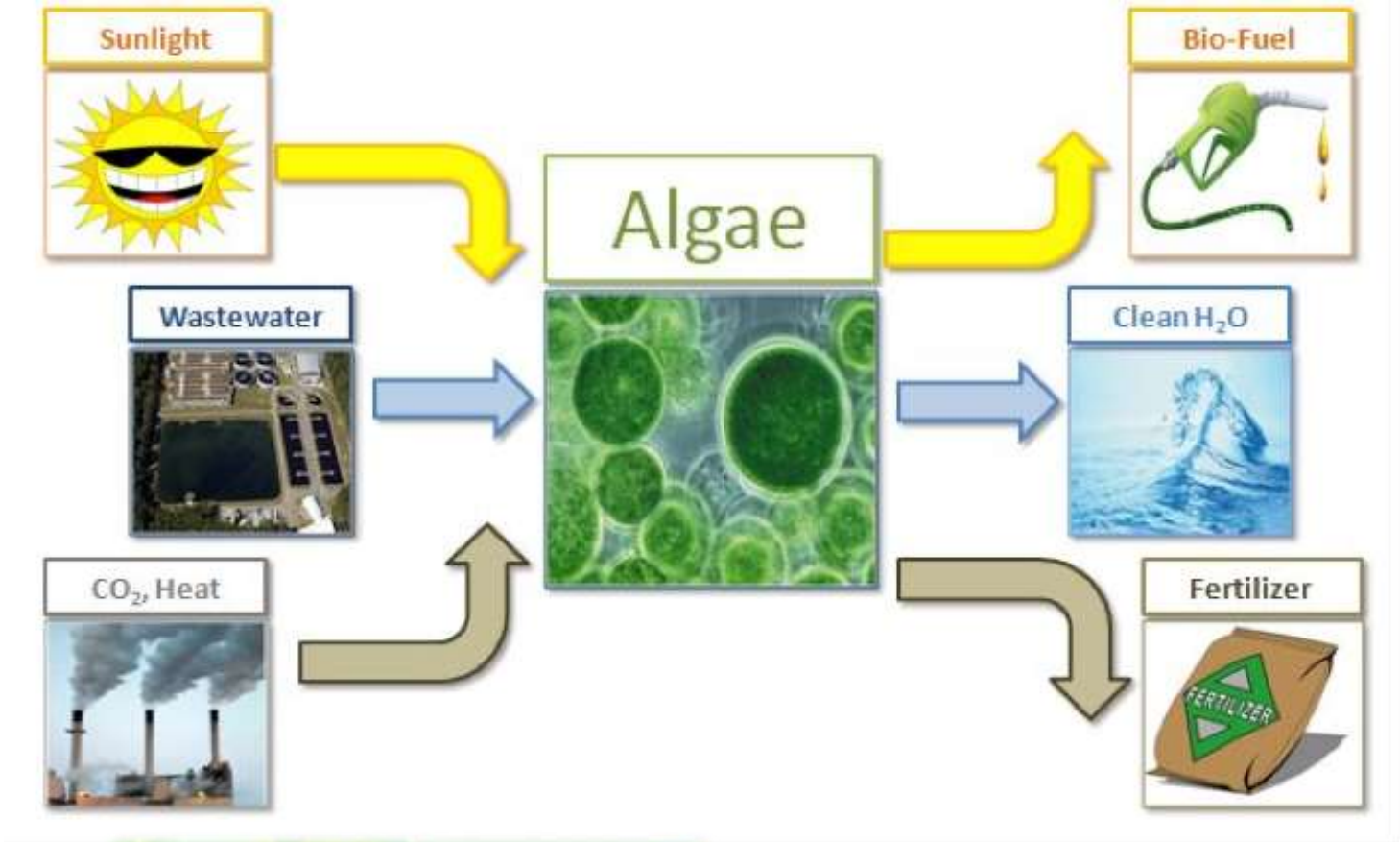
Epistylis coronata

- In a well-functioning activated-sludge plant, the protozoa community is dominated by **peritrichs** (***Vorticella* spp.**, ***Zoothamnium* spp.**, ***Epistylis* spp.**) and **hypotrichs** (***Aspidisca* spp.**, ***Euplotes* spp.**).

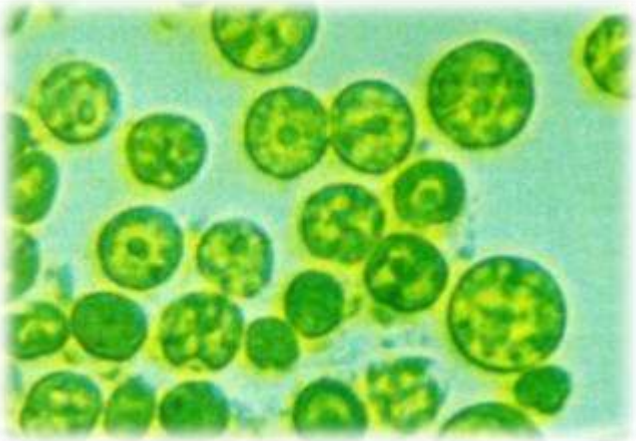
Algae

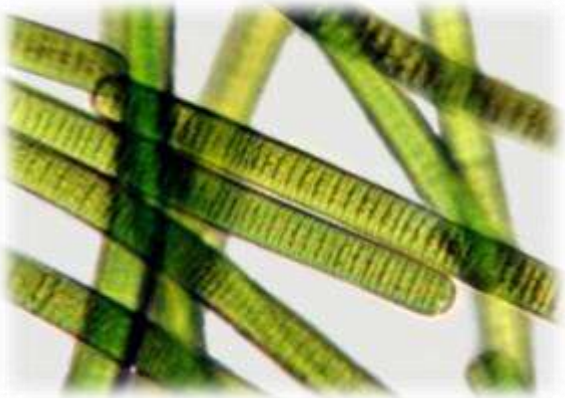
- ✓ Simple, eukaryotic, **phototrophic organisms** that carry out oxygenic photosynthesis using ***chlorophyll a*** - non vascular thallus
- ✓ In WW treatment systems, algae grow on the nutrients in the water.
- ✓ Found in highest numbers on surfaces such as trickling filters or walls of clarifiers, where the biologically treated waters trickle over the top of the clarifiers.
- ✓ Algae are significant organisms for biological purification of wastewater because they can accumulate plant nutrients, heavy metals, and pesticides, organic and inorganic toxic substances.
- ✓ May cause problems in clogging or eutrophic blooms after discharge of treated wastewater to receiving waters

- *Chlorella*, *Scenedesmus*, *Euglena*, *Chlamydomonas*, *Oscillatoria* *etc*.
  - ❑ **Chlorophyta** was dominant both in variety and quantity followed by **Cyanophyta**, **Bascillariophyta** and Euglenophyta.



Chlorella



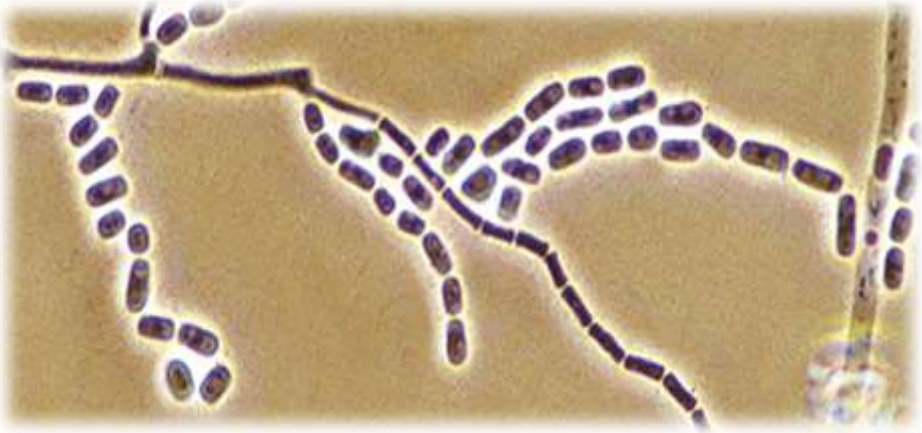


Oscillatoria

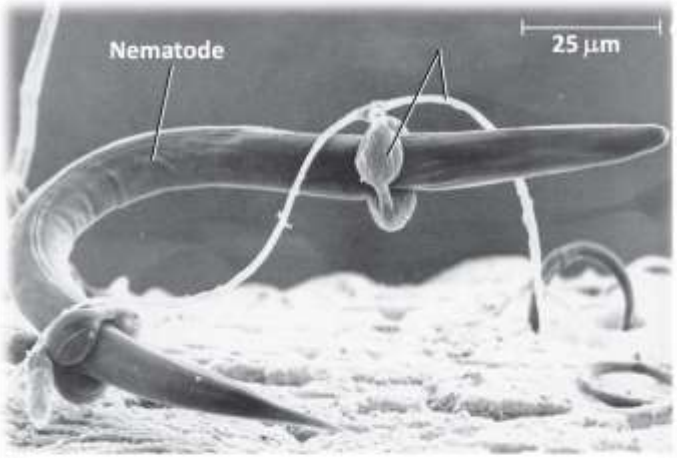
Fungi

- ✓ With the exception of yeasts, fungi are multicellular. Most reproduce with sexual and asexual spores
- ✓ They are chemoheterotrophs.
- ✓ In lower quantities than bacteria
- ✓ High hydrolytic potential
- ✓ Fungi have the ability to degrade cellulose, tolerate low nutrient levels, and grow in the presence of low moisture and low pH conditions.

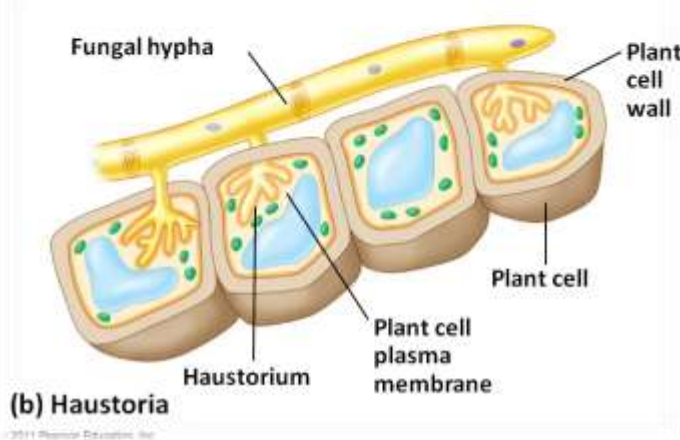
- ✓ The most frequent fungi colonizing fixed film processes are *Subbaromyces splendens*., *Ascoidea rubescens*., *Fusarium aquaeductuum*., *Geotrichum candidum* and *Trichosporon cutaneum*
- ✓ Fungi take part in the removal of the carbonaceous matter- the conversion rate- much higher than bacteria ,encourages a significant production of biomass
- ✓ To great proportion of fungi in the biomass favors the formation of a highly resistant biofilm that is difficult to slough.
- ✓ **Clog the aerated filters**
- ❑ Yeast can degrade organic compounds to CO_2 and H_2O



Geotrichum candidum showing chains of arthroconida



(a) Hyphae adapted for trapping and killing prey



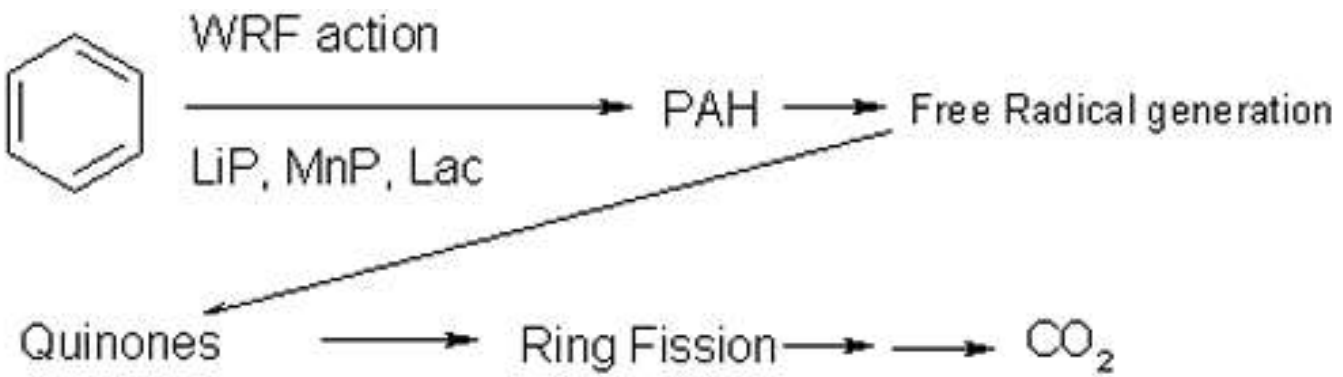
(b) Haustoria

Yeast can degrade organic compounds to carbon dioxide] and water with the use of free molecular oxygen (O_2), or as facultative anaerobes they can degrade organic compounds such as sugars to ethanol (CH3CH2OH) in the absence of free molecular oxygen.

- ✓ Excellent recalcitrant compound degradability
- ✓ **Dye degradation** by fungi -possibly due to the production of the lignin-modifying enzymes laccase, Manganese Peroxidase (MnP), and Lignin Peroxidase (LiP)
- ✓ White-rot fungi- most commonly used
- ✓ Effective in degrading complex aromatic organic compounds present in wastewater. For instance, phenolic compounds present in olive mill wastewater are similar to those derived from lignin degradation

Fungi - source of a variety of valuable biochemicals.

- ❑ Integrating wastewater remediation with recovery of valuable resources - economically viable solution for **sustainable waste management**



Rotifiers

- ✓ **'Wheel bearer'**-ciliated anterior (corona)- **LOCOMOTION**…..
- ✓ Multicellular invertebrates usually between 100-500 µm
- ✓ Mostly found in freshwater.
- ✓ Female dominant population- **Parthenogenetic reproduction**
- ✓ The rotifiers break up floc particles providing nuclei for new floc formation and they clarify the effluent by removing non-flocculated bacteria that are in suspensions- clarify the effluent by removing leftover bacteria, algae, protozoa.
- ✓ **Powerful feeders** and they contribute to floc formation by producing fecal pellets surrounded by mucus.
- ✓ The presence of rotifers at later stages of activated sludge treatment is due to the fact that these animals display a strong ciliary action that helps in feeding on reduced numbers of suspended bacteria (their ciliary action is stronger than that of protozoa).

More commonly found in activated sludge

The name "rotifer" is derived from the Latin word meaning "wheel-bearer"; this makes reference to the crown of cilia around the mouth of the rotifer.

- **The rapid movement of the cilia in some species makes them appear to whirl like a wheel.**
- **In most species, the head carries a corona (crown) of cilia that draws a vortex of water into the mouth, which the rotifer sifts for food.**
- **The food itself is ground by the trophi (jaws), located just behind the mouth in the pharynx (throat).**
- **]Trophi are found in almost all rotifers, and are characteristic organs of the phylum Rotifera.**
- **The body of the rotifer is externally but not internally segmented. The body is telescopic, with a semi-flexible, extendible, transparent cuticle covering.**
- **It is the cuticle that suggests rotifers are close relatives of roundworms and arthropods. Within the body are the stomach and reproductive organs**


Habrotrocha angusticollis
25 µ

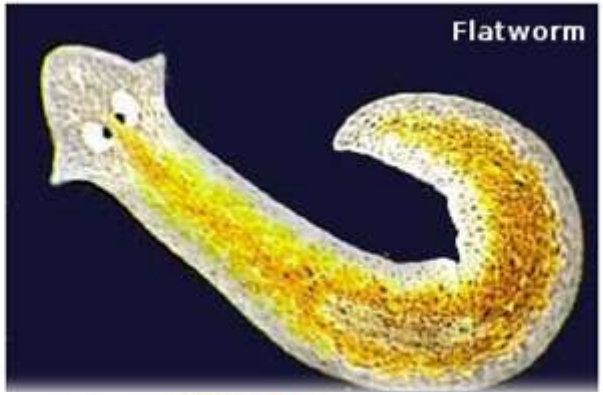

Philodina gregaria


Notommata allantois


Lecane arcula

Nematodes

- ✓ Roundworms, <1 cm long
- ✓ Move by Whip like motion.
- ✓ **More commonly found in trickling filter material.**
- ✓ Helminths -**Nematoda** (round worms), **Platyhelminthes** (flat worms) and **Annelida** (segmented worms).
- ✓ Feeds on bacteria, fungi, protozoa and sometimes other nematodes.
- ✓ Round worms and flat worms are endoparasites of humans as well as several animals. Eg; *Ascaris lumbricoides*
- ✓ The eggs released by the infected individual may reach water bodies and contaminate. *Taenia solium (port tape worm) and Taenia saginata (beef tape worm) and Schistosoma (blood flukes) species are important* pathogenic platyhelminthes.
- ✓ Helminth eggs are the main source of infection through water. Due to their relatively large size, helminth eggs are usually removed by processes such as sand filtration and sedimentation.



Psychoda larvae

Lumbricillus codensi

Tardigrades

- ✓ Tardigrades –also known as Water bears
- ✓ Water-dwelling, 4 pairs of short, stumpy legs with claws.
- ✓ Orange, red and green bodies
- ✓ They feed on algae and small protozoa
- ✓ They can survive extreme environmental swings
- ✓ They are very sensitive to toxic conditions
- ✓ They do not survive well in the presence of ammonia

One of the most resilient known animals

Meet the tardigrade, the animal that will outlive us all…..

Conclusion

- ✓ The main reason for treating wastewater is to prevent the spread of diseases by safeguarding water sources against pollution.
- ✓ Treatment of wastewater is one of the strategies for the **management of water quality**.
- ✓ Due to some drawbacks over the years concerning chemical treatment, biological treatment is now employed to avoid the unpleasant conditions in natural water resources.


THANK YOU