FARM MICROBIOLOGY 2008
PART 7: WATER & WASTEWATER MICROBIOLOGY

I. Water – General and Microbiology.
   A. Domestic use of water. Drinking, bathing, cleaning, formulating drugs, media-making (for bacteriology labs), etc.
   B. The water supply and the hydrologic cycle.

![The hydrologic cycle diagram]

C. Types of water. Surface, subsurface. Latter usually has advantage of having been filtered.

D. Definition of potable water. Simply water that is fit to drink. Contains no pathogens and an absolute minimum of other organisms. Also contains no harmful chemicals or anything that will prevent water from being used as it is intended to be used.

E. Drinking water. As it is provided to the public, it is made potable mainly by sedimentation, filtration or chlorination. UV light or ozone may substitute for chlorination.

F. The problem of wastewater. As it is discarded from whatever application in which it has been used (home, farm, industry, etc.), it may contain elevated levels of microorganisms (including pathogens), organic material and chemicals such as nitrates and phosphates. Organic material, nitrates and phosphates can serve as nutrients to assist proliferation of microorganisms in a lake such as bacteria (including cyanobacteria) and algae.

G. Water pollution.
   1. Sources of microorganisms in water. Soil, vegetation, animal and human wastes, decomposing animal and vegetable matter.
2. **Intestinal disease organisms spread by polluted water.** These include organisms causing typhoid fever (*Salmonella enterica* ser. Typhi – aka “*Salmonella typhi*”), diarrhea (E. coli, various serotypes of *Salmonella*), bacillary dysentery (*Shigella*), cholera (*Vibrio cholerae*), protozoan infections (amebic dysentery, giardiasis, cryptosporidiosis), viruses (poliomyelitis, hepatitis).

3. **What about Legionnaire’s disease?** Caused by the gram-negative bacterium *Legionella* which is water-associated in that it grows in cooling towers and large air-conditioning systems and is spread by aerosols. Generally not found in drinking water.

4. **Eutrophication.** Growth of algae and bacteria due to elevated levels of organic matter and chemicals such as nitrates and phosphates.

II. **Protection and Purification of Water.**

A. **Protection of water supply against sewage pollution.**

1. **Location and construction of wells.** This is overseen by the Wisconsin DNR. The **Wisconsin Well Code** is a set of regulations that is available as a pdf file at [http://www.legis.state.wi.us/rsb/code/nr/nr812.pdf](http://www.legis.state.wi.us/rsb/code/nr/nr812.pdf). An example: One cannot drill a well in one's basement as there is potential for flooding which can cause contamination of the well.

2. **Boiling.** A method mainly for individuals to prevent pathogens. Boiling will not kill endospores as a rule, but usually that problem is negligible in relatively clean waters.

3. **Chlorination.** Also designed to kill pathogens. Not much (if any) effect on endospores. Used to great effect as shown in chart below.

4. **Sedimentation.** Part of water purification in reservoirs.

5. **Filtration.** Note effectiveness in chart below. Basically consists of running water through sand filters. Also filters on taps.

6. **Ultraviolet irradiation.** Loses effectiveness with increasing depth of water.

![Effect of water purification on incidence of water-borne disease.](chart.png)
Special Topic: What is a Coliform?
(This may have been already covered elsewhere in the course.)

Coliforms are a group of easily-detected organisms in that their **gram-negativity** and **ability to ferment lactose** to acid and detectable **gas** can be exploited in the preparation of suitable selective-differential media that are relatively easy to make, inoculate and interpret.

Coliforms are generally associated with certain environments which can easily contaminate the water supply. Rather than test for individual intestinal pathogens in water, one can test for coliforms – the presence of which may indicate possible contamination by intestinal waste such as sewage. Hence they are called **indicator organisms**.

1. If *Escherichia coli* is isolated from water, then intestinal waste contamination is confirmed, as the habitat of *E. coli* is the intestinal canal of humans and other vertebrates.
2. Other coliforms (such as *Enterobacter* and *Klebsiella*) are often found in soil, and their presence in water can indicate surface runoff and the possible problems associated with it.

B. Potability testing.

1. **Test for nitrate.** Nitrate is poisonous above certain levels and also contributes to eutrophication.
2. **Test for coliform bacteria.**
   a. **Procedure.**

   (1) Inoculate a test tube of a broth medium containing lactose, a Durham tube (to trap gas) and inhibitory agent(s) against gram-positive bacteria. Inoculations can be made from dilutions of the water if a high degree of contamination is suspected. Coliforms would be expected to increase in numbers with the resulting population causing cloudiness and gas production in the tube. Subsequent tests include isolation of colonies on plates of an appropriate agar-containing medium and identification of coliform colonies to the genus or species level.

   (2) Water can also be filtered with the filter then placed on a solid medium in a petri dish; this medium can also inhibit gram-positive bacteria and (with an appropriate pH indicator) indicate colonies of cells which produce acid from the fermentation of the lactose in the medium. Colonies can be examined with identification made to the level of genus or species.

   b. **Interpretation of results.** Fermentation of lactose to acid and gas shows possibility of coliforms which can be confirmed or ruled out with further testing.

   c. **Standards.** When tested by the filter-plate method, generally there is a limit of one coliform colony allowed per 100 ml water sample.

III. Waste Treatment.

A. **Composition of domestic sewage.** Water, resistant compounds (including cellulose from paper products), colloids, protozoa, viruses, bacteria.

B. **Objectives of waste treatment.**

1. **Destroy organic material and reduce biological oxygen demand (BOD).** Amount of oxygen consumed by respiring bacteria in a closed container of a certain amount of a water sample can be measured and related to the concentration of oxidizable nutrients in solution. The less of these nutrients in water being discarded into the environment, the less of a problem with excess bacterial decomposition being evident.
2. **Remove components of fertilizers and detergents such as phosphates and nitrates.** These along with organic matter (above) are major causes of eutrophication.

3. **Kill pathogenic organisms.**

C. **General methods.**

1. **Disposal of untreated sewage (pollution).**
   a. **Dilution.** Dumping directly into rivers and various bodies of water
   b. **Irrigation.** This can lead to accumulation of bacteria, organic materials and chemicals in the soil which eventually get onto crops
   c. **Lagooning.** Letting sewage decompose in an open pond.

2. **Sewage treatment and disposal.**
   a. **Separation of solid and liquid (in general).** Screen out sticks, rags, trash – especially in large operations. Allow settling of material in suspension. Settling can be helped by some chemicals (e.g., aluminum potassium sulfate (alum), iron salts). Sediment can be incinerated or allowed to decompose more; liquid sent on for more treatment.
   
   b. **Septic tank (anaerobic) plus aerobic drainage system.** For small scale operations such as homes which are not hooked up to a municipal system. Note diagram below. Sludge is the solid stuff which has settled out and is acted on by anaerobic organisms. Carbon dioxide and hydrogen can be converted to methane by the methanogens. Sludge eventually has to be removed at intervals. Liquid contains a lot of bacteria and also organic material in solution. The organic material is oxidized by respiring bacteria as it runs out into the tile field.
c. **Large-scale operation.** Same general principles employed as above. Oxidation methods include **activated sludge** – large aerated pools where organic matter is converted by microbial activities to gases and inorganic compounds and also into the microorganisms themselves which form large masses called **flocs** (which can be removed and put into the anaerobic digester). Oxidation also occurs in the **trickling filter** where water is passed through a bed of gravel in which respiring organisms similarly decompose the organic matter. Effluent generally treated with chlorine and run through a long channel into a stream.

![Steps in sewage treatment.](image)

D. **Manure disposal.**

1. **National problem.** Confinement housing, very large feed lots. Great magnitude of the animal manure generated.

2. **Systems.**
   a. **Lagoons.**
   b. **Liquid tank (slotted floors).** (field disposal)
   c. **Anaerobic tank.** (field disposal)
   d. **Oxidation ditches.**
   e. **Stacking.** (field disposal)

3. **Problems.** Great potential for runoff and subsequent pollution of waterways. Problems with odor, flies, rodents. Public interest can lead to demands for guidelines, regulations and zoning.