13. Diseases of the Oral cavity and the Gastrointestinal system.

Resident "normal" microflora of the digestive tract.

Synopsis: The **oral cavity** has many resident types of microbes; bacteria, fungi and protozoa, most of the oral microbial population is bacteria, some of these produce acid from dietary sugar, which results in tooth **cavities** and some of them are involved in the formation of **tartar** and **calculus** deposits on teeth. Good dental health is essential, otherwise bacteria from the mouth may enter the blood circulation through dental cavities and is some cases can then cause a heart infection – **endocarditis**. The oral route can also be another way in which microbes gain access to other organs of the body including the brain, and those viral and bacterial infections of the brain can cause brain damage.
The **esophagus** is largely free of microbes, as is the **stomach** because of its highly acidic environment, many bacteria do survive the stomach and transit to the **small intestine**. There are many bacteria which will transit the small intestine but few of them will remain there except for the last third.
The **large intestine** has huge numbers of resident bacteria, up to 50% of fecal mass is bacteria of many species, but in which *Bacteroides* and *E. col*i tend to dominate. Food materials are resident in the large intestine much longer than in the rest of the tract, so there is a long time for microbial metabolic action and reproduction, some bacteria in the large intestine produce gas and acids, and some produce materials we can use, such as vitamin K and some of the B vitamins.

Bacterial diseases of the mouth:

Dental plaque, a coating of the tooth enamel composed of polysaccharide slime materials excreted by bacteria, especially *Streptococcus mutans*, it can be minimized by brushing and flossing and avoidance of sugars. A complex bacterial community builds up and lives in the plaque and one of the by-products is acid which attacks tooth enamel, leading to cavities.

Cavities are obvious cosmetic problems but they can also destroy teeth, which are needed for proper processing of food. Cavities may be an entry point for bacteria to the blood and can be the initial site where heart damaging bacteria gain entry.

The bacterial plaque will also coat teeth below the gum line, leading to gum inflammation. The milder form of this is **gingivitis**, which can become severe and result in gum inflammation, and ultimately **periodontitis** can occur which can cause tooth loss and bone damage in the mouth.

The common yeast *Candida* sometimes causes a white coating in the mouth and throat called **Thrush**. This is common in some newborns because they have not yet developed an active immune system, and is generally not anything to worry about given that the baby is under the vigilance of a nurse or doctor, but thrush can be one of the first signs that somebody who has an HIV infection is developing the first stage of active AIDS, because the immune system which prevents thrush in normal adults has begun to fail.

"Food poisoning"

In common public usage this term has a number of meanings, but in Biology 090b we have to be a bit more careful about how we refer to disease caused by interaction of microbes with the digestive tract. Which we are talking about - **food *poisoning*** or **food *borne infection***, makes a difference as to what strategy is best to prevent the problem or diagnose it if it has occurred.

Food Poisoning.

I will use this term to refer to situations in which there is bacterial contamination of food and those bacteria **grow** in the food and release **toxins** (poisons), which will exert their effect in the person who eats the food - in the digestive tract and elsewhere. Thus true food poisoning is an *intoxication*, not an *infection*.

In some cases it is possible to heat the food prior to serving it so that the toxin is destroyed along with the bacteria that produced it.

In other cases heating the food kills the bacteria that made the toxin but the toxin is NOT destroyed by heating, so that those who ingest the food will be poisoned. *Staphylococcus* enterotoxin is an example of a heat stable toxin that is produced by bacteria in foods and which is not destroyed by heating the food to boiling Heating *can* actually often destroy significant amounts of this toxin but since it only takes tiny amounts to make a person sick enough survives to cause food poisoning.

So, the key point with Food *Poisoning* is that it is bacteria that grow in the food **before it is eaten** that are the problem, they produce toxins and it is the *toxins* that cause the damage in the person who eats the food, and NOT DIRECTLY the bacterium.

Food borne infection.

In general public usage what I am about to discuss now is also referred to as food poisoning, but in the strictest microbiological terms it is NOT.

In food borne *infection* the major problem is that bacteria contaminate (and often grow in) the food and then those live bacteria are **transferred** to the digestive tract when a person eats the food. Once the bacteria are in the digestive tract disease begins. In some cases this is because the bacteria grow in numbers once they are ingested and *then* they release toxins, in other cases it may be that the bacteria are invasive and to varying degrees can cross the lining of the digestive tract, or lodge in it to cause damage.

USUALLY, in the case of food borne infection, if the food is heated to kill the bacteria there is no longer a danger to the person who eats the food, it may taste "off" perhaps, but under this definition it does not contain toxins and so is safe to eat after heating.

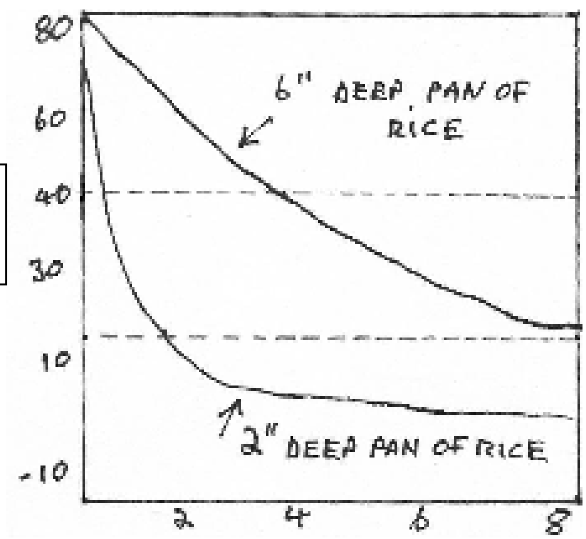
This leads to a universal dictum applied to the cooking of food:

Once food is properly cooked, cover it. Then do one of two things, keep it HOT enough so that no incident bacteria can live in it and reproduce, OR get it COLD - FAST! this will make the temperature too low for bacteria to multiply if they find their way into the food.

If you put cooked food out on the kitchen table, and then leave it to slowly cool down, you may set up a situation whereby little fingers get into the food when it is just at the nice warm temperature needed to allow contaminating bacteria to "take off" - to really grow rapidly in numbers and release toxins.

Look at a pan of cooked rice sitting on a stove top and left to cool down:

Temperature (Celsius)



Time (hours)

This graph shows the effect of the size of the pan and volume of rice on how long the rice temperature remains within the danger zone for microbial growth. The danger zone is between the hatched lines. Note how the line for the larger 6" container remains within the danger zone for hours, whereas that for the smaller container is only in the danger zone for a short period of time. In the 6" pan there is thus LOTS of time at the danger temperature range for bacteria to grow.

Bacterial food poisoning - specific examples

Staphylococcal enterotoxicosis. Some strains of *S. aureus* release **enterotoxins** into contaminated food, especially starch based or milk product laden foods. The toxin is heat stable and is not destroyed even if boiled for 30 minutes. Once in the intestine the toxin itself acts directly and the bacterium, if present, does not multiply but continues to release toxin.
The toxin causes pain (because the intestines are inflamed), diarrhea (largely because the toxin inhibits water and electrolyte absorption from the intestine), fever, vomiting (because the toxin stimulates the vomiting center of the brain) and is usually self limiting and does not require specific treatment. Avoidance of this disease is best accomplished by proper food handling procedures which sometimes has to involve identification of **asymptomatic carriers** of *S. aureus*.

Clostridium perfringens: I mention this organism because I noted that it is discussed in some of your other course materials. This is an endospore forming obligate anaerobic gram positive bacterium that also causes wound infections – gas gangrene. Food poisonings involving this organism often occur in meats or gravies, the toxin produced is released at the time when the bacterium is forming endospores. Poisoning by this organism is slower to appear, it lasts longer than Staphylococcal poisoning, and generally does not require active intervention, since it is usually self limiting.

Another bacteria that cause food poisoning that is rare but very dangerous when it does occur - **Botulism** caused by *Clostridium botulinum*. This gram positive endospore forming rod (bacillus) shaped organism will not grow in the presence of oxygen - it is a strict anaerobe. The organism survives as **endospores** in soil, so they may be commonly found on root vegetables for instance (as well as being endemic in lake waters in Canada). As long as those vegetables are processed properly there will be no problem, there will be enough oxygen around to prevent germination of the endospores, growth of the bacteria and production of the **botulinum** toxin (Botox as it is now commonly referred to). *Clostridium* food poisoning is primarily due to a toxin that binds with the "end plates" of neurons that activate muscles (these are called motor neurons) so that the muscle is paralyzed – a so called "flaccid paralysis" results, and this can be fatal if the muscles that are involved in breathing are affected. The risk of botulism is greatest when anaerobic conditions exist in food contaminated with spores of *Clostridia.* Curiously, although botulism is a result of a toxin carried in food, it does <u>not</u> produce appreciable <u>intestinal</u> discomfort, its major effect is on muscle activating nerves.

Washed fresh vegetables can be safely eaten. Food prepared according to the rule given above (keep it hot or get it cold - fast) will be safe from botulism. When WILL there be a problem?

1) Food being cooked by heating on the stove is contaminated with endospores, and it is allowed to cool slowly. If the food is in large enough volume it may be anaerobic at the centre (heating drives off the oxygen), and endospores at that point may germinate, bacteria will grow and produce botulinum toxin. This toxin is heat stable.

2) Food canning and bottling procedures are not done properly. If the food is acidic and prepared according to instructions (eg., acidified tomatoes) then there is little danger of problems with botulism. I believe that new instructions from governmental agencies in the USA now actually recommend that ALL foodstuffs are pressure canned or bottled, this includes such items as tomato's that have traditionally been acidified and treated by immersing in boiling water in bottles for home "canning".
If non acidic foods are processed and are NOT pressure cooked (like meats) then endospores will survive and may germinate. In that case one gets a "blown" can or a bottle which may gas off and will not seal - but sometimes **nothing wrong** can be seen, and this is a dangerous situation.

3) Improper storage of products like mushrooms which may contain contaminant endospores. For example, Do NOT store mushrooms in sealed plastic bags, aerobic

bacteria in the bags will use up all the oxygen in the bag which then allows anaerobic conditions in which the Clostridial endospores can germinate. You will notice that when mushrooms are plastic wrapped for sale in supermarkets, the plastic has lots of holes in it! Years ago, Natives in the far north were poisoned in the same way when well meaning but ignorant bureaucrats forced them to seal dried fish in plastic rather than leaving it dry and uncovered.

Note added: Some of you will remember the episode in October 2006 of botulism in bottled carrot juice, so botulism is rare but it happens! Several victims were paralyzed here in Ontario. If the patient can be maintained alive long enough the paralysis can be reversed and the patient will recover, but this is a long difficult process and fatalities are a real threat. Carrot juice is made from a root vegetable, which in my opinion demands extra care because there is more likelihood of contaminating endospores. Also, carrot juice is not acidic, as is the case with bottled citrus fruits, and this acidity somewhat protects against endospore germination.

Bacterial enteritis caused by food borne infection.

In this situation, bacteria directly inflame the lining of the intestinal tract, toxins are not directly involved, the bacteria multiply and adhere to, or invade the lining and may actually cross it into deeper tissues, depending on the causative organism and local conditions.

Sometimes the inflammatory process interferes with water and ion absorption across the intestine and the result is watery feces - **diarrhea**. If the process also occurs in the large intestine a severe diarrhea may occur which is called **dysentery** and may also involve destruction of the lining, causing the diarrhea to contain blood and pus. In some cases the organisms that have caused all of this may invade more deeply and cause other systemic problems, including **enteric fevers.**

Salmonellosis. (*S. typhi, S. choleraesuis, S. enteritidis*). Most *Salmonella* now grouped in *S. enteritidis*.

Many hosts exist for *Salmonella*, including the intestine of poultry, wild birds, rodents, and in poultry *Salmonella* may be deposited on to the egg shell or into eggs. Most cases of salmonellosis are a result of the sort of improper food preparation and preservation we have talked of above.

In salmonellosis abdominal pain, vomiting, and fever appear along with diarrhea which may be mucoid and bloody. The *Salmonella* bacteria commonly produce fevers when their cells lyse in the digestive tract and release fever inducing endotoxins (**pyrogens**) from their cell walls. In usual cases antibiotics are not given.

NOTE that water from municipal supplies and wells has been known to be contaminated with *Salmonella*.

[[There was a recall of products manufactured by Hershey in Smith Falls Ontario in November of 2006, because of contamination of the product by *Salmonella* that was alleged to be in material delivered by a supplier to the manufacturing plant. No actual case of salmonellosis was reported, but low levels of *Salmonella* had been found in product released to market. Late reports indicated that the salmonella was present in Soy-lecithin used in chocolate manufacture. As of November 2006 there were also reports of importation of cantaloupes from Mexico and the USA that were contaminated with *Salmonella* though no illnesses were reported. *Salmonella* contamination on this product has been an issue for a number of years and there is still uncertainty as to how the cantaloupes become contaminated. And, note added in 2008, we all should be mindful of the serious Salmonellosis outbreak in food service at Western, the actual origin of which was never discovered**]]**

Typhoid Fever - often just called typhoid. (*Salmonella typhi*).
Not a problem in Canada but a huge problem worldwide. Most generally caused by contaminated waters, often after heavy floods or natural disasters, but also spread in food. The bacteria enter the lymph tissue and are spread around by it when phagocytosing cells attempt to kill the bacteria but fail to do so, and they act as mobile hosts to the bacteria which are released by the phagocytosing cells to the circulatory system. The bacteria spread and grow in the blood, causing fever, headache, and then reach the intestinal lining, which they cross to be passed out in the stools. In most cases in otherwise healthy individuals the disease is not fatal and convalescence occurs in 3-4 weeks. Chloramphenicol is the drug of choice to treat the infection, though there are resistant strains. It is known that *S. typhi* can survive and grow in the gallbladder (in the bile) and be shed from there into the feces by non symptomatic carriers and this is one way in which the infection can be spread.

Shigellosis. (*Shigella* species). This is not as invasive a bacterium as *Salmonella*, though it commonly houses plasmids which code for toxin production, and *Shigella* has been known to pass these plasmids to other bacteria (such as the *E. coli* strain which caused the deaths at Walkerton). Even in situations where good sanitation is practiced (clean water, no fecal contamination from animals etc) it is possible to get shigellosis because people pass on the bacterium to others especially when they do not practice good hand washing, poor hand washing practice is probably the commonest way in which shigellosis is contracted.
Shigellosis is spread in food and contaminated water, children are more susceptible, especially in crowded conditions such as in day care centres. The bacterium attaches to the lining of the small and large intestine and cause cramps, profuse bloody diarrhea, and fever. *Shigella* produces a neurotoxin (**Shiga toxin**) and releases endotoxin when it lyses. The infection in children requires active intervention, much of the recuperating effort coming from steps to end the large fluid and electrolyte loss because of the high volume of diarrhea.

Cholera - most common in Asia. (*Vibrio cholerae*). Has very high mortality rates when epidemics occur which overwhelm health authorities ability to keep up. *V. cholerae* is water borne and is also found in contaminated food. *Vibrio cholerae* has been found to attach to the shells of shrimp, crabs, and shellfish such as oysters. The bacterium produces an enterotoxin that causes a copious watery diarrhea (“rice-water stool”) resulting in rapid and potentially fatal dehydration and salt loss. Treatment with **oral replacement therapy** is simple and dramatically successful in many cases, a simple sugar and salt fluid mix is given to patients to stop the fluid loss and re-establish proper ionic conditions in body fluids.

Other gastrointestinal diseases are caused by **viruses**. Examples are **Rotavirus** (these are reoviruses with a double stranded RNA genome) and **Norwalk** virus (these are calciviruses with a single stranded RNA genome). Rotavirus has not been a big problem here in Canada, but kills millions of children in developing countries. Norwalk virus has had increased incidence in Canada especially in places like homes for the aged and has been a major problem on cruise ships. Norwalk virus is self limiting and of short duration but causes intense diarrhea and vomiting. Just recently (October 2006) Mt Allison University in New Brunswick was actually closed for 3-4 days because of an extensive outbreak of what was believed to be a Norwalk virus infection with many students becoming sick, an outbreak also occurred at the same time at the University of Saskatchewan. In November of 2006 senior citizen homes and the hospital in Central Newfoundland were closed temporarily due to a Norwalk virus outbreak, and at the same time a cruise ship in the Caribbean returned to port because of a ship-wide outbreak of what was probably the Norwalk virus, that was tracked to having been “delivered” to the ship by a couple who were actively sick when they got on board the ship. This is the big problem with cruise ships, they concentrate people in a small area and in close contact and this is a superb “incubator”, since you are “locked in”.

Travelers diarrhea (aka Montezuma’s revenge, Tijuana foxtrot, Delhi Belly etc)
This is often caused by pathogenic strains of *E. coli*. In many cases what WE find to be pathogenic to us, is NOT to the local population. Caused also by other genera of bacteria (*Salmonella*, *Shigella*, etc) and by protozoa (such as *Entamoeba*) and parasitic animals. Travelers diarrhea and related intestinal infections are also caused by viruses and parasites;
There are numerous protozoan parasites that cause travelers diarrhea, such as dysentery caused by the amoeba – *Entamoeba.*

There are many strains of *E. coli* - genetic variants of the same species, which are often a result of the acquisition of plasmids which carry genes for toxins and other factors such as antibiotic resistance. Some carry a plasmid that holds a gene which allows the bacterium to adhere more persistently to intestinal cell surfaces, others carry a plasmid which holds genes for enterotoxins.

E. coli 0157:H7 is a special case that we may deal with later - it is the organism that caused the Walkerton situation.

In many cases no antibiotics are given for bacterial travelers diarrhea, but agents are given to soothe symptoms and stop the diarrhea.

There are sometimes long term sequelae to travelers diarrhea, it can take years for these later problems to resolve - like irritable bowel syndrome, and lactose intolerance - which can be permanent.

In foreign countries the causative bacteria are on food, carried on the bodies of people, in water, and it is difficult in some cases to avoid contact. Protective measures include frequent hand washing, not eating raw and uncooked foods, only drinking treated water and so on, but even all these measures diligently applied may not prevent the problem.

Ulcers of the stomach, esophagus or duodenum are often caused (70-95% of them) by a particular bacterium, *Helicobacter pylori*. Antibiotic treatments will frequently cure such ulcers. *H. pylor*i is also implicated in stomach cancer.

Hepatitis. There are a number of types of this viral liver disease (A,B,C,D,E) and they vary in severity and consequences. In many cases the hepatitis infections are mild to severe but complete recovery occurs, in some cases this does not happen and there are long term problems ranging from a high rate of liver cancer in hepatitis B to chronic disease and cancer with Hepatitis C. There are vaccines only for hepatitis A and B and in some cases such as B, C and D there are carrier states. Hepatitis is not a disease <u>of</u> the intestinal tract, but it IS frequently contracted as a food borne infection, although some hepatitis viruses are sexually transmitted, some are transmitted in blood (the hepatitis C “scandal” in Canada is an example), and some, such as Hepatitis A, are transmitted from affected people via mouth and feces and into food. Hepatitis **A** is usually mild, as is hepatitis **B** from which most recover though it is more severe than hepatitis A. Hepatitis **C** can be severe though most do recover, although it is the leading reason for liver transplants. Hepatitis **D** is severe and can be fatal when co-infection occurs with Hepatitis B. Hepatitis **E** is common in Asia and is especially dangerous for pregnant women.

Protozoan infections of the gastrointestinal tract.

Giardia (aka Beaver Fever), worldwide, but also a problem in Canada - in places such as Newfoundland. Caused by the protozoan *Giardia intestinalis*. Found especially in surface waters, such as lakes and ponds, it is spread in the feces of animals such as the beaver. *Giardia* produces dormant and environmentally resistant cysts which can survive chlorine treatment, it causes a copious diarrhea, inflammation, malabsorption syndrome etc. In some cases it causes protracted problems which take months to resolve. Treatable with antiprotozoan agents such as metronidazole and quinacrine. The problem is avoided by drinking treated water. Some communities in Newfoundland are under long term “ boil water” orders while water is treated with iodine, since chlorine is ineffective against cysts when used at acceptable levels.

Amoebic dysentery is caused mostly by *Entamoeba histolytica*, it is common in tropical climates. *Entamoeba* produces **cysts** which are the usual way they are ingested by humans. The organism is found in food and water, and can be passed on by fecal contamination, and it will invade across the gut wall as well as causing just diarrhea, so one can get damage to other parts of the body - and have a chronic state of the disease.

Cryptosporidiosis, Cyclosporiasis - both have now been reported in Canada, the infections can be acute and painful but usually self limiting, though occasionally one can get serious chronic problems. "Crypto" has been a problem in municipal water treatment systems in Canada, and "cyclo" has been found on imported produce because of improper watering and washing procedures in the country of origin.

Fungi

Fungi cause problems as producers of toxins in foods. We don't find diarrheal disease caused by fungi (as far as I know) but do have numerous fungi which release toxins into foods.

Aspergillus. – this fungus produces the **aflatoxins**, which can cause liver cancer. Especially a potential problem in peanuts and grains, there are now strict controls in Canada for this fungus problem. The **ergot fungus** *Claviceps purpurea* grows on rye and wheat and releases toxins such as ergot (also the basis of the first effective anti-migraine drug) and other toxins having effects including hallucinations, fever, abortion, and sometimes, death. **Poisonous mushrooms.** Not a problem unless you have the stupid habit of eating wild mushrooms without a VERY clear understanding of the risks. *Amanita* is the primary genus containing fatally poisonous mushrooms.

Helminths (parasitic "worms").

Flukes - the **liver fluke**, can be resident in animals such as sheep which excrete it into waters where snails house it until it forms larvae, which encyst when shed into water, from where humans can contact them. The cysts activate, bore through the intestine and lodge in the liver and elsewhere in the body. The **Chinese liver fluke** is the best known example - and there is no effective treatment, clean water and good sanitation practices are the best preventatives.

Tapeworms. These are "egg laying machines" which attach to the intestine and can grow to as long as 20 + feet, they can absorb so much nutrient from the host digestive tract that the infected person can suffer from malnutrition. Tapeworms release huge numbers of small "packets" called <u>proglottids</u> containing thousands of eggs, and these are released in feces. There are various types of tapeworm in a range of animal vectors. General avoidance is by clean water, good sanitation practices, but some drugs can be used successfully.

Trichinosis. A roundworm infection which is commonly present in temperate climates like ours, and the infections it causes are usually a result of improper cooking or handling of infected pork products, and is caused by encysted larvae that survive in body tissues of pigs. The cysts can be killed by thorough cooking of pork products. Trichinosis is very rare in Canada, there are now strict laws which require cooking of swill to kill the parasite. Untreated infections cause growth and residence of the worms throughout the body, and treatment is symptomatic only - there is no cure.

14. Applied Microbiology

Soils are very complex materials chemically and physically, they are often layered and there are many types. Soils hold huge numbers of bacteria, fungi, viruses, protozoa, microscopic animals, in a dazzling variety of combinations and numbers, all of them form very complex ecosystems that interact with each other and the world at large, indeed, the world is dependent on microbial ecosystems for development of healthy soils so that plants grow, for recycling of dead plant and animal materials to make them available again (decomposers perform this function), for the involvement of many microbes in the mineral cycles we mentioned earlier. Much of the dead organic matter on land ends up in the soil and is decomposed by bacteria and fungi and also protozoa and microscopic or small animals, this renders the organic material into a friable (crumbly) state which contributes to the structure of soils and also much of the organic material is decomposed into basic simple inorganic molecules such as carbonates, sulfates, nitrates, nitrites, nitrogen, ammonia which can then be assimilated by plants.
Animals cannot digest the huge tonnage of cellulose found in plants, this task is carried out by bacteria and fungi and some protozoa, in the guts of herbivorous animals (deer, horses, cattle ants etc) and termites, and much cellulose breakdown occurs directly by soil and forest floor and leaf litter dwelling fungi and bacteria. Methane is a major byproduct of the microbial breakdown of cellulose.

Microbes are found in the air, huge numbers of fungal spores are in the air. There is a growing “mini-industry” for microbiologist-consultants, airborne microbe analysts who look for microbes in room and industrial air that may be causing illness or allergies. Politically speaking this has become a “hot issue”, many are worried about “**sick building**” situations, where the sealed nature of many new buildings may “lock” people inside with air contaminants that they feel are making them sick. Sometimes these contaminants are chemicals like volatile glues and paints, but some microbes may cause problems.

Molds may grow in moist areas like poorly designed and maintained ducts and damp construction materials like wall-board. The molds may release many spores into the air that some people are allergic to. Certain “black” molds such as *Stachybotrys* are known to release volatile toxins into the air that can harm babies and others. Airborne microbial problems can be minimized by proper design of buildings, avoidance of areas of consistently high moisture that allow good fungal growth, and good air exchange with the outside. High efficiency **filter** systems are used in some buildings to remove contaminants and in some cases confined air, as found in small rooms used to inoculate microbes or that need to be kept clean for other reasons, may be irradiated by powerful UV lamps that are turned on when people are not present, to kill microbes.

Microbes in water:

All natural water bodies contain microbes, the numbers and types depend on the chemical constituents, temperature, oxygen content and pH of the water. Some of the microbes in natural waters may be pathogens. Upper levels of ponds and lakes and rivers will have photosynthesizing microbes, such as cyanobacteria and algae, these add biomass to the water system by deriving energy from sunlight. One can also find photosynthesizing bacteria in waters that do not use chlorophyll as the light energy capturing pigment, but use a purple pigment, and in the lower regions of waters within the anaerobic depths of mud and silt, are anaerobic methane producing bacteria. Each type of water body - freshwater lake, or pond or river, saltwater – shallow or deep, very cold or very hot water, has its characteristic forms of resident microbes. Microbes also drain into natural water bodies from the land, or are washed in by rain.

Water pollution is a major political and social issue and takes many forms, from farm field runoff of animal wastes and chemicals, to industrial pollutants of many kinds, mining runoff and discharge, sewage, etc as well as natural materials such as silts and sediments originating from upstream floods, deforestation, and ice and snow melts. In many cases the natural microbial composition of waters is disturbed by factors such as chemical pollutants that kill certain types of microbes, or by runoff of nutrient rich waters from farm fields that encourage overgrowth of certain types of microbes at the expense of others.

In some cases the huge increase in microbial numbers caused by large nutrient pollution inputs to the water can deplete the oxygen in the water very rapidly, because microbes use the oxygen while they metabolize these pollutant nutrients, and this can kill off fish that needed the oxygen, algal blooms can do this. Massive algal and cyanobacterial blooms can then release toxins into waters that can kill fish, make water taste “off” and in some cases the toxins are harmful to people. Heat is a pollutant in this sense, warm waters, perhaps released by a power plant, will encourage microbial growth to speed up and cause oxygen depletion.

When a nutrient enters a water body as a pollutant, it provides nutrition to microbes, and in using that nutrient they will use and deplete the dissolved oxygen in the water. Thus we have the term Biological Oxygen Demand (**BOD**) that is used to describe the pollution status of waters. When a water body has a high BOD value (determined by a special test) this means that the water will support rapid microbial growth and can lose oxygen rapidly – possibly causing a fish kill for instance. BOD test values are thus an <u>indicator</u> of the degree of pollution of waters with materials that encourage Heterotrophic microbial growth and consequent oxygen depletion. Waters that are overloaded with such nutrients, compared to more pristine natural waters, are described as being **eutrophic**. In the process of eutrophication the water will support heavy growth of aquatic plants and may well produce algal and cyanobacterial blooms which will cause a depletion of dissolved oxygen.

Of course, in some cases pathogens are washed from agricultural soils and industrial processes, as well as human sewage facilities, into water bodies, from where they can find their way into waters used for drinking or cooking purposes and can infect people. The classic case we will deal with later is the appearance of the toxic strain of *E. coli* from cattle, that appeared in Walkerton's drinking water because of a crack in the casing of one of the town wells.

Fecal coliforms are rod shaped gram negative bacteria (*E. coli* being the classic but not sole example) normally resident in our large intestines or the intestines of animals such as farm cattle, pigs etc.

The presence of fecal coliforms in water is indicative of contamination of the water from a source of animal or human feces, fecal coliforms are thus **indicator** bacteria. *E. coli* is <u>the</u> usual **indicator organism** for fecal contamination of our municipal drinking water supplies, the reasonable assumption is that if one finds fecal strains of *E. coli* growing on agar or other test media inoculated with a sample from a town or well-water supply, then that water supply has been contaminated somehow with fecal matter that would also carry other potentially pathogenic organisms of fecal origin, thus *E coli* is an <u>indicator</u> organism for fecal contamination.

Note once again that not all coliforms are *E. coli*! There are a number of other bacterial genera that are in the coliform group, and are found as colonizers of human and animal intestines, such as *Klebsiella.* Remember, also, that many coliforms are <u>not</u> disease causing and are a normal component of our gut microflora, and are also commonly found in soils.

There are numerous chemical tests for water quality, and microbiological tests such as **total plate counts**, along with specific agar plate counts for specific kinds of bacteria can be performed. A statistical test for numbers of coliforms and fecal coliforms has been used for many years, the most probable number (**MPN**) method, using serial dilutions of water samples in broth containing tubes. Larger volumes of water sample can be passed through a **membrane filter** that retains bacteria, the filter is then placed on a pad of nutrient medium so that colonies grow and can be counted. Special colour indicator tests are also used, where special nutrient broth solutions are inoculated with samples and specific colour reactions indicate the presence of coliforms.

Treatment of municipal water supplies:

Municipal potable (drinkable) water supplies are first treated with **flocculating** agents such as **alum** (potassium aluminium sulfate), these will bind with suspended matter and sediment it into a sludge "blanket" so that it sinks and can be removed, the water is then filtered through sand beds. **Chlorine** is used to kill microbes. Often the chlorine is injected into the water as a gas. The chlorination process must be carefully monitored, chlorine will bind with organic matter in the input water (such as tannic acids from decayed leaves around lakes and rivers) and enough chlorine must be added to bind with all of the organic matter in the water and still have enough **free** (unreacted) chlorine left over to kill bacteria (this is called **residual** chlorine), but only just enough to do this.

The chlorine must be dosed so that it will do this job of killing microbes both in the treatment plant and in the piping and distribution system to the end user – the homes connected to the system, but so that there is virtually no free chlorine left at the end users tap – all of this takes good training of operators so that they do the tests properly and know when there is a problem and know what has to be done about the problem. In some cases there are pathogens that cannot be effectively treated with safe levels of chlorine. An example is the cysts of *Giardia*, which cause nasty intestinal problems. The cysts are resistant to chlorine, and boil water orders must be issued while the system is analyzed, and then generally the system must be treated for several months with iodine. In some cases bad odours, colour, and "off" tastes are removed from drinking water by filtration through activated carbon, since this is not (contrary to the belief of many) accomplished by chlorine.

Sewage treatment:

Human wastes (and animal wastes in intensive farm operations) must be processed. There are two major reasons for treatment. Raw sewage wastes cannot be released into waters because they impose huge BOD loads on them which will kill fish and render the water body eutrophic or even stagnant. Most of this BOD load is a result of the fecal solids, and these waters must be reduced in BOD load by conversion of the wastes into microbial biomass solids (instead of fecal solids) in the secondary treatment system, this resulting sludge can then be collected and disposed of by burning or land fill burial. The second reason is that sewage wastes contain pathogenic bacteria, viruses and other organisms such as protozoa. In short, the treated waste water produced from the input sewage has to be as free as possible of pathogenic microbes, and must be severely reduced in its BOD load before it is released into lakes, streams or rivers. It is impossible to sterilize the huge volumes of treated sewage wastes.

There are three levels of sewage treatment - primary, secondary and tertiary. **Primary** treatments remove solid materials such as paper, plastic, metal, oil based material etc and grease, these are settled out by addition of special flocculating chemicals and/or are skimmed out by special mechanical skimmers. After primary treatment the **secondary** treatment process begins, the waste water is sent to trickling filters or activated sludge systems. Both of these have the same aim, soluble and insoluble organic matter (feces and urine) in the waste water is converted into clumps (floc's) of solid organic matter in the form of the bacteria, fungi and protozoa that are produced by growth on the nutrients in the waste water. The organic soluble load of the waste water that would normally flow away, has now been converted into solid microbial biomass that can be removed because it can be physically settled out of the waste water flow. It is in the secondary stage of sewage treatment that most of the BOD is removed. The vast majority of municipal secondary treatment systems are aerobic.

In the case of **trickle filters** the waste water is introduced to the top of a tall tank that is full of hollow plastic or ceramic rings or large (2 inch) pieces of crushed rock. As the water trickles though the tank to the bottom, bacteria grow, using the nutrients in the waste water, these stick to the rock and accumulate to form slime layers, these slime layers feed on the wastes in the water as it trickles by and thereby clean it. Periodically the slime layer dies and sloughs off and falls to the bottom of the tank where it is collected and removed, new slime layers form where the old layer sloughed off.

Activated sludge (**AS**) systems are based on the same principle as trickle filters, formation of solid microbial biomass from soluble waste so that it can be removed. But, AS systems are continuous, operate at high rates and can handle much larger volumes of waste than trickle filters. AS systems continuously introduce wastewater into a large tank where lots of air is pumped in, encouraging rapid bacterial growth, the sludge that results consists of floc's of clumped bacteria and fungi that form when these organisms use the soluble and solid waste input as food, thus the waste material is converted into microbial biomass floc's that are suspended in the exit flow that is continuously removed to a settling tank.

The floc's settle in the settling tank to form a sludge and this exits at the bottom of the settling tank, the treated clarified waste water that has been freed of the floc's is removed at the top of the tank on a continuous basis. Ideally, the process is designed so that the sludge consists of floc's of matted bacteria, and bacterial and fungal filaments of the right size for rapid settling, a small part of the sludge is continuously <u>returned</u> to the AS tank from the settling tank as a seed for continuous formation of the floc's of microbial biomass back in the main AS treatment tank. Sludge removed from this secondary treatment is compressed to remove water and then either burned or sent to landfill sites for burial.

Tertiary treatment is often referred to in the trade as "polishing". It is costly and is often not done, soluble materials that survive secondary treatment (such as phosphates and nitrates) are removed chemically, excess ammonia is removed by air stripping, and the waste stream may be treated with chlorine, UV light, ozone, or other agents to kill surviving microbes or destroy viruses.

Home **septic tanks** are based on the same principle as secondary treatment plants, conversion of house wastes into solid microbial sludge's that can be removed periodically, but they are less efficient, and unlike municipal secondary treatment systems, septic tanks also probably rely more heavily on <u>an</u>aerobic bacterial activity to treat sewage wastes. Further waste decomposition takes place aerobically when the remaining waste water is discharged from the septic tank into the weeping tile for delivery to the leaching field in the soil in back yard of the house, soil microorganisms complete the decomposition of wastes at that point. Homeowners must be careful not to discharge toxic materials (home chemical wastes such as solvents and bleach etc) into the septic tanks since this will perturb the microbial population and damage its sludge forming activity. It is also important to pump out the accumulated sludge on a regular basis (yearly every two years etc, years depending on regulations and local conditions), if this is not done then untreated waste survives in large amounts into the leaching field.

An extension of this principle of using microbes to clean waste water is **bioremediation**, a growing industry in which bacteria and sometimes fungi, often specially developed, are used to clean polluted soil or water. Oil spills and kerosene spills can be treated with bacteria that "eat" the material and convert it either into water soluble materials that can be removed or into microbial biomass that can be removed. Oil "eating" bacteria were sprayed on to oil covered rocks after the Exxon Valdez oil spill in Alaska in 1989, with some degree of success. Bacteria are injected into areas of soil containing contaminants. An example is the injection of solutions containing bacteria into sites in the US where the armed forces have left stockpiles of explosives like TNT that have leached into and contaminated the underlying soil, the bacteria use the TNT residue as food and remove it. Bacteria can also detoxify dioxin and PCB contaminated sites. The white rot fungus *Phanerochaete chrysosporium* is very good at destroying complex organic molecules such as creosote, and PCB's and has been used to clean up sites contaminated with these substances. Bioremediation works, but it is generally slow, incomplete, and expensive.

Food and Beverage Microbiology

Microbes have been used by humans for food and beverage manufacture for millennia. Of course, until about 150 years ago humans did not KNOW that microbes existed, but they <u>were</u> being used for food preparation and industrial purposes. Bread, cheese, yogurt, wine and beer making are all essential and ancient processes and they involve fungal and bacterial components.
Microbes had other uses than associated with food. For example, in ancient times a lot of clothing was made of flax, and the fine soft fibre was prepared by placing the raw fibre with its tough sheathing into a stream and submerging it using rocks to weight it down, for months, this rotted away the harsh fibres and left the fine flax fibres, this process is called **retting** and it is actually accomplished by the action of a microbe present in the water – a species of *Clostridium* – a bacterium.

<u>Food</u>.

Sometimes there are microbial components of food that are not desirable. In some cases this is because the food already harbors the microorganisms, in other cases they <u>arrive</u> and multiply because of improper storage and processing, in some cases <u>normally</u> resident microbes multiply and become a problem because of improper storage and processing.

Grains at one time were commonly infected with the ergot fungus *Claviceps purpurea*, mentioned earlier or *Aspergillus*, these fungi produce dangerous toxins and testing is necessary as well as proper storage, with dry conditions free of vermin. Bread products are susceptible to a number of molds such as the common bread molds *Rhizopus*, *Neurospora*, *Monilia*, *Penicillium*, *Aspergillus*. Bread flours can also be infected by spores of *Bacillus* bacteria, and if these survive baking, they can germinate and grow and rapidly make the bread cheesy and ropy in texture and this can be a difficult contaminant to remove from a bakery.

Fruits and vegetables, especially those which have been contacted by soils, often harbor the soft rot bacterium *Erwinia carotovora* and this will cause a soft squishy slimy rotting, especially with improper moist and overly warm storage – this was a common problem in root cellars. Many fruit and tomatoes (which, of course are fruit too!) are infected by *Pseudomonas* bacteria. In class I mention the Irish Potato famine causing fungus *Phytophthora infestans*. This latter fungus is one of the huge number of primary plant pathogens – pathogens that attack and damage or kill crop plants in the field, this is just too huge a subject to be dealt with in this course, and this includes the dominant crop damaging wilting fungi *Verticillium* and *Fusarium*. Many gastrointestinal pathogens may be incidentally associated with fruits and vegetables, by their presence in the soil, or because these were in irrigation or wash water. Yeasts and some other fungi, and bacteria that contaminate fruit juices can result in the inadvertent production of alcoholic beverages though they are not likely to be very palatable!

Meats and Poultry. Much of the microbial load on meats comes from improper handling or inadvertent cross contamination from their digestive tracts when the animals were gutted and cleaned after slaughter and there are a wide variety of contaminant organisms. These are mostly bacteria, though some fungi can cause problems. It is much rarer for there to be microbial problems from presence of microbes in the tissues of the living animal itself, though meat and poultry does sometimes harbor bacteria not derived by spillage from the gut (like *Mycoplasma*). If animals, especially pigs, are fed with tapeworm or other worm infected feed (trichinosis is the common example) the meat itself can contain infective parasites or their cysts or eggs, though thorough cooking will kill them.

It is also known, though thought to be a rare problem, that prion (BSE and CJD) infection can occur with contaminated meats, especially from the spinal cord, there are now strict rules as to what can be fed to cattle and how they are to be slaughtered in order to minimize the problem of prion diseases. With poultry, a source of *Salmonella* is eggs, they can acquire the bacteria in the oviduct before the shell has fully formed. There are a number of bacterial infections transmissible by meat that has been prepared improperly or cooked improperly, these include anthrax, brucellosis (which can also include spontaneous abortions and is caused by the bacterium *Brucella abortus*), Q fever which is caused by a very small obligate bacterial parasite of beef, and listeriosis, caused by the bacterium *Listeria monocytogenes* – which has also caused large scale food borne infections from contaminated cheese.

Of course, of particular newer and serious importance are infections of meat by the highly toxic strain *E. coli* 0157:H7. We will look at this later.

More risk of high microbial contamination is associated with ground meat products such as hamburger than would normally be the case for non ground “whole cuts” such as steak and roasts, this is because surface contaminating bacteria on the meat used for hamburger production become mixed throughout the hamburger, and it is possible for the bacteria to grow in higher numbers because they now have access to finely ground beef which has been broken down into more accessible components. There is also a higher risk of high microbial numbers in and on the apparatus used to form hamburger if the machinery is not routinely and thoroughly cleaned.

Fish and Shellfish can be heavily contaminated with microbes. Thorough cooking and use of fresh fish (or fresh frozen, canned or dried fish) both solve this problem. Shellfish are filter feeders, so they will concentrate fat soluble toxins from their environment, this can include toxins accumulated that are produced by certain algae they eat, this has caused problems in Canada – such as paralytic shellfish poisoning (PSP) outbreaks. Some bacteria fix strongly on to shellfish shells, such as *Salmonella* and *Vibrio*. There have been outbreaks of botulism in the far north because of improper preparation and drying of fish that harbor endospores of *Clostridium botulinum*.

Milk. Centuries ago drinking milk could be a risky business, but in many cases it was more dangerous to drink water! Milk is usually microbially clean before it leaves the udder of healthy cows. Milk is not likely to be sterile at that point, there are some normally resident microbes in the teat region that find their way into milk before it is released, there are numerous ways that microbes can find their way into milk as soon as the milk is released from the udder, the teat will contaminate the first small volume of milk as it is released, and microbes on hands or on improperly cleaned milking equipment can end up in the milk and there are opportunities for entry of microbes during storage and delivery. Many of the bacteria normally found in milk are not pathogenic but may cause the milk to become ropey or slimy, or taste bad, or to curdle.

There are some pathogens that can be found in milk from some sources, cattle are tested now to ensure that the TB organism *Mycobacteria* is not present (though this is usually the milder bovine strain), and a bacterium called *Brucella* can sometimes be present that causes spontaneous abortion in cattle and can cause disease (undulant fever) in humans, though these are not common now since cattle are vaccinated against these organisms. **Cheese**, which is of course a milk product, can harbor harmful bacteria largely as a result of contamination during improper processing, *Listeria* is a common example.

Honey. There have been cases of botulism in babies who ingest honey that contains spores of *Clostridium botulinum*. This results in a <u>flaccid paralysis</u> called the "floppy baby syndrome". Very young babies can be at a stage where the protective antibodies obtained from their mother, in the milk colostrum, are disappearing, but their own immune system has not yet matured , this causes a short "window' of time where they are more vulnerable. Also, in very young babies there is not a full and complex "mix" of bacterial species colonizing the large intestine, that can attack *Clostridium* bacteria and eliminate them or at least exert competitive pressures that restrict their growth. Both these factors, the lack of a fully formed microbial gut ecology and the undeveloped immune response can expose babies to danger from honey.

<u>Prevention of food spoilage and transmission of disease in food.</u>

We will not have time to go through all the myriad varieties of microorganisms in or on food, some harmful – pathogenic or spoiling, some not. All forms of pathogen can be transmitted in or on food, bacteria, viruses, fungi, protozoa, animals and as we now know – prions in meat. Our purpose here is to discuss ways in which these infectious agents (with the probable exception of prions) can be killed to prevent pathogenesis and to prevent or slow down food spoilage.

Canning (or bottling) is a common domestic and industrial practice for food preservation. Where **endospores** are of concern autoclave temperatures <u>must</u> be used for a sufficient period of time to be certain that the full temperature penetrates to the innermost region of the food for the correct amount of time, endospores can survive boiling for extended time periods, hours in some cases, meats must be subjected to this higher degree of heat treatment for instance. In other cases **natural acidity** or <u>added</u> acidity, in combination with heating to boiling temperature, is sufficient to accomplish sterility, tomatoes are a classic example, natural acidity supplemented with vinegar (acetic acid) or citric acid (lemon juice for instance) and boiling are sufficient to obtain "sterility". Actually, the use of the word sterility in this case is more in the sense of the public vernacular use of the word, acidified tomatoes that have been subjected to boiling water baths may contain endospores that have NOT been killed, but they will not germinate and grow in the acidic conditions, and in this case, in strict scientific terms, the tomatoes are not sterile.

One of the commonest signs of failure to sterilize foods like tomatoes or soups is "<u>flat sours</u>", thermophilic ("heat loving") bacteria survive and they are of a kind that does <u>not</u> cause cans to bulge by creating gas, the food becomes sour tasting and unusable. In other cases contaminated and improperly treated cans will contain live bacteria that have grown from surviving endospores, and these bacteria will grow and produce gas and cause the cans to bulge. This latter case is usually caused by *Clostridia* or other anaerobes and is referred to as thermophilic anaerobic spoilage (aerobic spoilage cannot occur in properly sealed cans, there is little or no oxygen present unless the can is breached or improperly made).

Pasteurization is NOT sterilization, it is a milder form of heat treatment used principally on milk and fruit juices etc to reduce the microbial load so that they stay fresh longer. It kills some pathogens but not all of them. Flash pasteurization occurs at about 72 C (71.6 C to be precise) for 15 seconds, conventional pasteurization occurs at about 63 C (62 .9 C to be precise) for 30 minutes. In Europe, milk can also be purchased that has been heated to higher temperatures, and has a longer shelf life than pasteurized milk. This ultra high temperature (UHT) milk product has a slight but pleasant caramel after-taste and was developed years ago because refrigerators were too expensive in Europe. Evaporated and condensed milk in cans was originally developed for the same reason.

Some foods are preserved by **osmosis**. Jams contain high sugar levels and salted fish has high salt content. In both these cases it is the hyperosmotic condition of the food relative to the cytoplasm of microbes that "sucks" the water out of bacteria and fungi by osmosis so that they cannot grow, and this is an ancient and efficient means of food preservation. Some organisms do grow in these conditions though, especially some fungi, there are some yeasts that can grow in high brine conditions and will form creamy white material in brine preserved pickles, and some fungi can grow on the surface of jams but do not tend to grow extensively down into the jam.

Drying foods is another common and useful way to preserve them, as is shown by the common home based practice of slicing materials such as apples and tomatoes thinly and drying them at only warm temperatures.

Refrigeration (at about 4 C) retards the growth of many, but not all microbes for a matter of days. Freezing (-10 to –20 C) can suspend the metabolism of microbes and can kill some of them, but many survive freezing. It is an effective way to preserve food, but not all foods can be preserved by freezing, they lose their texture. Some bacteria are psychrophilic (they grow best at cold temperatures), but these are rarely agents of human disease. BUT, the pathogen *Listeria monocytogenes* is an example of a pathogen that can grow at refrigeration temperature!

Freeze drying (**lyophilization**) requires special vacuum refrigerated devices and these are expensive and are only used for special items such as instant coffee or dried yeast, but freeze drying is very effective, it does not necessarily kill microbes, but they cannot grow since they are almost completely devoid of water.

Irradiation of foods is very effective, gamma radiation penetrates food containers effectively, is highly lethal to microbes and gives a long shelf life to irradiated food, but there are some strong public objections raised against irradiation and it can be a politically tricky issue. Some of the objections arise out of basic ignorance of science. Many people seem to believe that radiation "sticks around" in the irradiated food. This is not true of course, there is no residual radioactivity in irradiated food. Many also erroneously believe that irradiated food becomes radioactive. **Gamma rays** are powerful, killing, and can penetrate deeply. They have been used to irradiate fish, vegetables (so that they do not sprout), and fruit. In some cases irradiation might cause an off taste in foods because it can chemically alter some food constituents, like fats. **Ultraviolet** light is of little value, and this is because it does not penetrate foods to any great degree though it can be used to irradiate surfaces used in food preparation. As mentioned, **Microwaves** can be used to kill microbes because the heat they generate kills them, but home microwaves should not be used for this purpose.

Chemicals are added to foods to preserve them. Antioxidants are added but are not used as direct agents to retard microbial degradation of foods, sugar and salt are chemical antimicrobial chemicals, and numerous gases are used to fumigate foods to retard microbial spoilage – these include chlorine and sulphur dioxide. Nitrates and nitrites are added to meats like sausages now largely to redden them for public tastes and also because they retard microbial growth (nitrites are known carcinogens).

Addition of antibiotics is not a good way to preserve food, and is rarely done, for good reasons, some people are allergic to them, and problems of generalized microbial antibiotic resistance would be worsened. Also, the legal ability to use antibiotics at will would almost certainly make people lazy in practicing good general hygiene.

Microorganisms as food, and used in food production.

Microbes used directly as food? Algae and fungi are classed as microbial organisms but actually can form macroscopic (large) structures and these have long been used as food. Algae is used in the form of Nori, Dulse and a number of other products and can be an important dietary supplement for iodine. Agar is derived from algae and besides the obvious use in microbiology, has been used extensively in baking. Carageenan is another algal polysaccharide (from Irish Moss – which is an alga, NOT a moss!) that is used extensively as a texture enhancer in foods, in milk products like ice cream, puddings, candies etc and it is used to form stable foam heads in some beers, and is also found in toothpaste. Mushroom are fungi and a variety of them are important food products around the world.
There was extensive research in the 1960's and 70's towards using cheap processing waste products from sugar processing or making corn syrup etc to grow huge amounts of yeast as food products for the poor in developing countries - so called SCP (**single cell protein**). It became clear that such foods contain too much nucleic acid that can damage people (it causes gout, kidney and other problems because of an overload of nitrogenous waste) and it was too expensive to remove it.

Microbes are greatly involved in food production.

Bread, wines, beers etc are produced by the action of yeast (though some of the alcohol and some of the particular tastes are a result of bacterial action in some cases). The yeasts responsible for alcohol production and rising of bread dough are <u>all</u> strains of the yeast *Saccharomyces cerevisiae* which forms alcohol and carbon dioxide as waste products of anaerobic (non oxygen requiring) metabolism of glucose. **Bread** forms when yeast is mixed with water, flour and other substances such as fats, oils, sugar etc and kneaded to form dough. The dough is left to rise, which occurs because many millions of tiny bubbles of carbon dioxide are formed, by the yeasts, as they fermentatively (in the absence of oxygen) metabolize the sugars in the dough. The dough is then baked. Yeasts used in baking are either moist cake yeasts or freeze dried yeast pellets. Some ethanol is produced when bread dough is formed, along with the carbon dioxide bubbles, but baking evaporates it.

Yeasts are an important component of food and beverage production economics.

Sourdough breads involve a bacterial fermentation as well as the fermentative yeast action mentioned above. The bakery maintains a "mother sponge" – a starter culture which contains the yeast (*Candida milleri*) and a lactic acid bacterium (usually *Lactobacillus sanfrancisco* or a similar species). The *Candida* yeast, unlike *Saccharomyces*, can use glucose in the dough, but not the maltose, the bacterium can use the maltose but not the glucose so they do not compete for nutrient and the bacterium produces lactic acid and acetic acids that give the tart flavour to the bread, while the yeast produces the carbon dioxide that causes the dough to rise. Sourdough breads are often made in Europe from rye flour because yeast alone does not do a good job of leavening the dough, because rye flour is low in gluten and is "harder" but the lactic acid and other acids produced by the bacterium partly degrade and soften the dough so that it rises better.

There are significant industrial processes devoted just to the production of yeast for bread-making and for wine and beer production. In the case of bread and baking yeasts the yeast may be in a semi moist "cake" form or it may be formed into hard dried tiny yeast pellets. The term "fermentation tank" often applied in descriptive literature to the large tanks where the yeasts are produced in huge commercial operations is misleading. Fermentation means no oxygen is used, but those "fermentation" tanks are heavily aerated. The yeasts that will be dried or formed into cakes and shipped to the supermarket or sent to commercial bakeries are grown using molasses and other additives.

If you grow *Saccharomyces* in the presence of large amounts of sugars, they will NOT want to grow in the aerobic manner (using oxygen) that gives maximum numbers of yeasts, which is what is desired in commercial yeast factories, instead, they exhibit what is called the **Crabtree effect** which means that if high amounts of sugar are present then no matter how high the oxygen levels are, the yeasts will preferentially undergo fermentation and <u>not</u> use the oxygen that is present, this is a slower less efficient process that produces a lot of unwanted alcohol and much less yeast cell yield. So, instead, the sugar levels are controlled to a lower constant level that still provides all the sugar the yeast needs and huge amounts of air is pumped into the tanks so that the yeasts grow aerobically and thus in maximum yield, those "fermentation tanks" do NOT undertake fermentations!

For **beer** and **wine** the yeast is the same as that used in bread making, but the strains are optimized for production of alcohol. Beer is produced from grain seeds (barley usually) that are sprouted and dried, this causes the grains to produce enzymes that will degrade the starches in the seeds into sugars when the sprouted seeds are mixed in warm water – this is called the **mash**, which then forms a rich mixture of nutrients called **wort** when hops and sugar are added. Yeast is added and fermentation begins, this produces alcohol (ethanol) and lesser amounts of other alcohols that impart aroma and flavour, the yeasts are either floating and are skimmed off or they sink and are pumped away – this depends on the type of yeast and the process, the remaining beer is aged, filtered and pasteurized before bottling.

Wine properly speaking is a grape product, although the word is now used for beverages produced by other fruits, or honey (mead) by fermentation. Sulphur dioxide is used to kill undesirable wild yeasts, the grapes are crushed and yeast is added (many different strains of yeast are used depending on the wine). After fermentation the wine is siphoned free of grape skins (already removed if it is a white wine) and sediment, it is allowed to age, and is them clarified, bottled and stored. **Spirits** ("hard liquor") are produced by distillation of these primary fermentations to concentrate the alcohol, this is done with grains (whisky, rye), potatoes (vodka), rum (molasses) etc, the alcohol content is 40 to 50 % versus 6% for beer or about 12% for most wines.

Numerous **milk products** are formed by microbial action (including fermented alcoholic products using mare's milk), principally **cheeses** and **yogurts**. The bacterium *Lactobacillus acidophilus* is an " acid loving" (acidophilic) bacterium that grows in and curdles milk (causes the lactose sugar containing **whey** to separate away from the precipitated protein which is mostly casein), this bacterium is responsible for formation of yogurt and *Streptococcus thermophilus* and other acid producing bacteria may also be involved., buttermilk is formed commercially by addition of *Streptococcus thermophilus* to milk.

Cheeses are formed by action of a curdling enzyme taken from calf's stomach (**rennin**, now genetically engineered to be produced by a bacterium) and/or use of **lactic acid bacteria**, whose principal role is to sour and curdle the milk, the whey is separated out by pressing and the curds are the basis of the cheese. Some of the lactose sugar of the milk is thus converted to lactic acid by the lactic acid bacteria and this adds to the efficiency of the curd forming process. Lactic acid bacteria do not usually function in long term ripening of cheese to form the aged cheeses such as cheddar. Soft cheeses are not ripened once the whey has been pressed out, these include ricotta, cottage cheese, cream cheese and they do not have a long shelf life. Harder cheeses with longer shelf lives are **ripened**, this involves aging of the cheese either by addition of special microbes that impart flavour or microbes that are naturally present accomplish the formation of the particular kind of hard cheese, these microbes are in the body of the cheese under the forming rind. In some cheeses veins of fungal hyphal growth form (*Penicillum roquefortii* for example) and are visible as strands and pockets throughout the cheese as blue or greenish blue sections, Blue cheese, and Roquefort and Stilton are examples, and these fungi release pungent compounds into the cheese that give it the characteristic taste. Some fungi will grow in hard cheeses while they mature but are not desirable, such as *Geotrichum* species which impart a "sweaty socks" flavor to cheese!

The mycelial fungus *Aspergillus oryzae* functions in fermentation of **soy** to produce products such as soy sauce, and production of miso, tofu and sufu involves other fungi such as *Mucor* .

The <u>Lactic acid bacteria</u> mentioned above are very important components of the formation of a number of milk products such as cheeses, yogurt, fermented goat and horse milk products (kefir and koumiss), these bacteria are mostly species of *Lactobacillus* and *Streptococcus.* The lactic acid bacteria are also involved in the fermentations that produce sauerkraut and pickles. Lactic acid bacteria are also responsible for the ripening processes in meat products such as salami which undergo a mild fermentative maturation. Also, the healthy antibacterial low pH environment of the vagina is at least partly maintained by lactic acid bacteria.

Other microbe involving food products are; vinegar, sauerkraut, pickles, olives, poi, Soy sauce, Soy products such as Miso and Tofu, fermented meats. The mycelial fungus

Aspergillus oryzae functions in fermentation of **soy** to produce products such as soy sauce, and production of miso, tofu and sufu involves other fungi such as *Mucor*

Many industrially useful products are made by microbes, and ethanol is a good example made industrially in a number of ways, but mostly by yeast fermentation of corn sugar in Canada and the USA, but other methods are being developed, some of which use bacterial fermentation, such as use of *Zymomonas*, much of this research was done here in Canada, and perhaps the worlds greatest expert on high alcohol yield industrial fermentation is based in Canada, William Ingledew – in Saskatchewan. Industrial alcohol is used as a solvent and as a base chemical in formation of many other industrial products. Butanol and acetone are important industrial solvents and base stocks that can be made by fermentation of sugars by the bacterium *Clostridium acetobutylicum*, glycerol used in pharmaceutical products and food and cosmetic manufacture is made by a yeast fermentation.

Microbes produce enzymes with various medicinal and industrial uses such as proteases, amylases, ligninases etc. Microbes also produce vitamins, dietary supplement amino acids, hormones and other pharmaceuticals.

Of course, lets not forget the genetic engineering of microbes to produce materials such as human interferon, insulin, growth hormone etc.

Microbes are also used to clean up pollution, as we mentioned when looking at bioremediation, and in some cases microbes are used to solubilize metals like copper, iron, zinc lead etc because they create acids that dissolve these metals and the fluids can be recovered and the metals separated