JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - III ISSUE - III MAY-JUN 2010

Editorial

Contents

Well to start the issue for these months, the section on Mini Review takes a look at the different facets of Microbes and Fermentation. Fermentation has been known for many decades as a specialized process, which initially may have surfaced as a process to prevent spoilage. The article also makes a note of the different parts of the fermentor, which are either an important or optional requisite to the fermentation process depending on the type of fermentation, the microbes so employed and the criteria that is to be met in different fermentations. The article looks at different fermentation processes and provides a brief description of the raw material that is used in various fermentations.

In the history of medical care the 'concept of asepsis' may be regarded as the most important landmark of advancement. As far back as nearly two centuries ago, microbes were established as the causative agents for disease and infections. This discovery obviously necessitated precautionary measures to keep medical material free from possible microbial contamination prior to clinical use. This protective step is known as 'Sterilization', which is defined as 'complete' destruction and removal of all forms of contaminating microorganisms from the materials concerned. In Current Trends we delve into Radiation as a means of sterilization.

Jules Bordet's early studies showed that antimicrobic sera include two active substances, one existing before immunization, known as alexine, and the other a specific antibody created by vaccination: he developed a method of diagnosing microbes by sera. He discovered haemolytic sera and showed that the mechanism of their action on foreign blood is similar to that by which an antimicrobic serum acts on microbes and, furthermore, that the reactions of the sera are colloidal in nature. Thus Jules Bordet is known for his studies on humoral immunity and is In Profile for this issue.

The Bug of the Month, *Bacillus cereus* food poisoning occurs year-round and is without any particular geographic distribution. The short-incubation form is most often associated with rice dishes that have been cooked and then held at warm temperatures for several hours. The bacterium also produces a highly heat resistant endospore which allows the bacterium to survive under hostile conditions.

There are several compounds that are used for antisepsis and disinfection and these include iodine and its compounds. Though less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they're often associated with irritation and excessive staining. Did You Know draws attention to the preference of PHMB over iodine compounds such as povidone iodine.

Some tests, such as bioburden or viral titer tests, are quantitative in nature while other tests, such as those for the presence of objectionable organisms, are qualitative. As with chemical tests, these differences necessitate different validation approaches. The purpose of a test also may change the procedures for running and validating it. Therefore Validation of Microbiological Test Methods is important and is mentioned in Best Practices.

Relaxation is a must and the same is provided for a perfect blend of information on a lighter note.

We look forward to hearing from you, your suggestions and feedback helps us in our endeavor to keep you informed and updated.

Microbes and Fermentation

Microbes have, from the beginning been a part and parcel of the earth and have indeed existed before us, evolution has pushed them to survive adverse and changing conditions, though they may be pathogenic culprits, they also comprise a non interfering commensal population, and there are of course quite many that serve beneficial purposes to mankind, like those involved in antibiotic production, decomposition, fermentation etc.

Microorganisms are capable of an amazing array of fermentations. Most natural compounds are degraded by some type of microbe and even many man – made compounds can be attacked by bacteria. In environments devoid of oxygen (or other suitable inorganic electron acceptor), this degradation involves fermentation.

Fermentation dates back many decades, of understanding this desirable process and the practice of using the same for a beneficial and yielding process.

Fermentation is defined as an energy yielding process whereby organic molecules serve both as electron donors and electron acceptors. The molecule being metabolized does not have all potential energy extracted from it. In other terms, it is not completely oxidized.

Brief Summary of Fermentation Requisites

Despite the many methods bacteria employ to ferment organic compounds, there are some unifying concepts that are true of all fermentations.

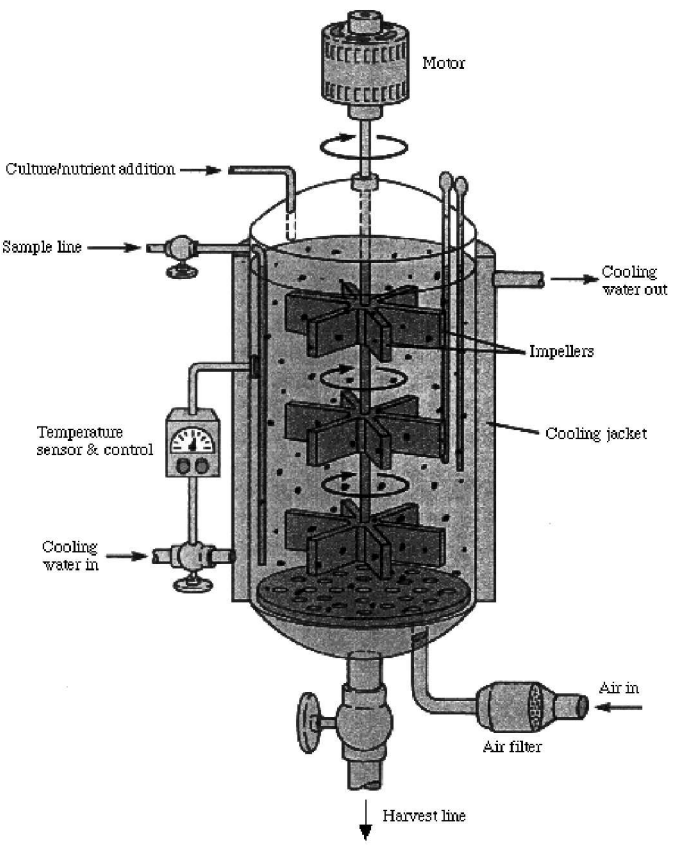
1. NAD+ is almost always reduced to NADH
   The electrons are removed from the organic molecule and most often given to NAD.
2. Fermentation results in an excess of NADH
   Accumulation of NADH causes a problem for anaerobes. They have too much of it and prevents further oxidation of substrate due to lack of an NAD+ pool to accept electrons. In many fermentation pathways, the steps after energy generation are performed in part to get rid of the NADH.
3. Pyruvate is often an important intermediate
   Many of the reactions eventually end up in making pyruvate. Pyruvate is an valuable intermediate because it can be used for cell synthesis and many different enzymes can act on it. It gives the microbe flexibility.
4. Energy is derived from Substrate Level Phosphorylation (SLP)
   The substrate is converted to a phosphorylated compound and in subsequent reactions the high energy phosphate is transferred to ATP.
5. Energy yields are low
   Substrate Level Phosphorylation (SLP) is an inefficient process and much of the energy of electrons is lost. Typically energy yields are 1 to 4 ATP per substrate molecule fermented.
6. Oxygen is not involved
   Fermentation can involve any molecule that can undergo oxidation. Typical substrates include sugars (such as glucose) and amino acids. Typical products depend upon the substrate but can include organic acids (lactic acid, acetic acid), alcohols (ethanol, methanol, butanol), ketones (acetone) and gases (H2 and CO_2).

Fermentations of Importance to Humans

For millenia humans have taken advantage of the fermentations microbes perform. Over the years humans have learned to control and optimize these fermentations through trial and error. It was only in the last 100 years that the biochemistry behind fermentation has become clear. The production of microbial products is called fermentation but it is not fermentation in the defined sense of the word.

Parts of the fermentor

Fermentor operates on a combination of various principles, however each and every part of the fermentor play a pivotal role in the outcome of the fermentation and fermentation products.



Vessel: fermentors or Bireactors are specialized vessels that are used for the growth of microorganisms on a small or large scale. Industrial fermentors can hold upto 200,000 litres of culture. Fermentor and all pipework must be sterile before fermentation begins. This is usually achieved by flushing of the whole system with superheated steam before the production begins. The vessel that may be used can range from a simple bucket, jar to an ideal fermentor, usually made out of stainless steel as many fermentations produce acid. The vessel is usually of a symmetric

shape like a cylinder, to ensure that there is uniform mixing and concentration of media, etc. Depending on the type of fermentation that has to be carried out, quantity of culture, the product and by product of the fermentation, the material of the fermentor may vary. However there are some characteristics of the vessel that must be fulfilled in order to make the vessel ideal for use in a particular fermentation.

Other conditions that must be satisfied include:

- Cells have to be provided with nutrients in a very carefully controlled environment to keep them in desired growth stage.
- Nutrients and other materials have to be fed in by valve operated pipelines.
- Conditions in the fermentor have to be carefully monitored to regulate cell growth.
- If process is an aerobic one the fermentor has to be well aerated.
- Incoming air is filtered and pumped into the base of the fermentor – a valve releases the pressure from the top of the tank.
- An antibiotic producing fermentation may use a tonne of sugar a day.
- Most fermentation are batch processes eg. Penicillin, beer and wine.
- Nutrients and the innoculum are added to the sterile fermentor and left to go on with it.
- Anti – foaming agent may be added.
- Once the desired amount of product is present in the fermentor the contents are drained off and the product is extracted.
- Some products are made by a continuous culture system.
- There is a steady stream of nutrient input, and spent medium is drained off.

Though not many products are made this way, however certain manufacturers and processes may be more beneficial by this method.

Shaft: Is a rod like, usually fitted in the center of the vessel on which the impeller blades are fitted, and these are uniformly fitted on the shaft. At the bottom of the shaft there may be a sparger which aids in aeration.

Impeller blades: If the culture is thick or sticky additional stirring is required by a motor driven paddle called an impeller. As mentioned these are fitted on the shaft and bring about mixing of the media, culture microorganisms and nutrients. These have to be made of an appropriate size, depending on the diameter of the fermentor vessel, in order to bring about uniformity.

Sparger: This is required in cases of aerobic fermentation processes. As is depicted in the figure, the sparger may be in the form of a plate at the bottom atomizing air packets into the medium. The size of the holes for dispersing the air in the medium also vary with the type of microorganism used.

Sterile air supply: Fermentations may be of the aerobic or the anaerobic types, In cases when the process is aerobic there is a need to supply sterile air. In order to achieve this there is a reservation made to continuously supply sterile air to the medium.

Sample line: Before and during the fermentation process, it is important that the medium as well as the product quality be tested. And for the same purpose, a sample of the fermentation broth is taken and analyzed primarily for contaminating microbes, etc.

Cooling jacket: While initially the culture may need warming to start of the process – once it has started a cooling system is vital. The organisms are likely to raise the temperature of the culture by more than $1^{o}C$ per hour; more heat will come from the activity of the impeller. This rise in temperature could quickly kill the microbes if not cooled.

Cooling is achieved by either a water jacket or cooling coils inside the fermentor. Fermentation is very often a heat generating process and the same heat may be lethal to the microorganisms used in the process, in order to cool the fermentor vessel, there is a cooling jacket which has cool water passing through, thus the heat of the vessel gets absorbed by the cool circulating water and the fermentation regains its appropriate temperature, monitored by the temperature probes.

Motor: The rotation of the shaft and impeller blades is brought about by the motor, therefore is responsible for the speed at which the medium is agitated during fermentation.

Nutrient addition system: As the process of fermentation advances, there are some nutrients that get exhausted and it is important to provide certain vital nutrients, especially in cases when auxotrophic mutants (a mutant strain of microorganism that will proliferate only when the medium is supplemented with some specific substance not required by wild-type organisms) are used then the additional supplement has to be monitored and added as required.

Harvest line: When the fermentation has yielded the required product, it is through the harvest line that the product is obtained.

Interior of the fermentor is monitored by sterilizable probes which record temperature, pressure, stirrer speed, pH, oxygen and carbon dioxide levels,

for instance;

Temperature probe: Are probes that are inserted into the medium to monitor the temperature of the fermented broth, in case when the temperature exceeds the favorable temperature there are mechanisms like the cooling jacket that aid in maintaining the correct temperature.

pH probe: During many fermentations there is acid that is produced, which can be lethal to the employed microorganisms, hence when there is a pH fluctuation away from the desired pH a small amount of base/acid is added.

Major Pharmaceuticals and Biotechnology Industry Applications

There are 5 major groups of commercially important fermentations:

1. Microbial cells or biomass as the product, e.g., single cell protein, bakers yeast, *lactobacillus, E. coli* and many others.
2. Microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase and others.
3. Microbial metabolites:
   - Primary metabolites – ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides and others.
   - Secondary metabolites – all antibiotic fermentations.
4. Recombinant products: eg. Insulin, HBV, interferon, GCSF, streptokinase.
5. Biotransformations: Phenyl acetyl carbinol, steroid transformations and others.

Commercially Important Classical Examples of Fermentations

Brewing Beer

Beer is a fermentation of barley and hops by yeast. The starch and barley is broken down into glucose and then fermented to ethanol by yeast. The finished product is aged and then packaged for distribution and consumption. It is a six stage process, beginning with the formation of malt from barley.

Steps involved in Beer Fermentation

Malt – Barley is first soaked in water for 5 to 7 days. At this time the grains germinate and produce amylases (enzymes that degrade starch to glucose) and proteases (enzymes that break down proteins). These enzymes are essential to the brewing process. Amylase provides sugar for the yeast fermentation and the proteases solubilize compounds in the grain and hops important for the quality of the beer. The germinated malt is then dried and crushed.

Mash – Mashing solubilizes the starch and other flavors in the grain and extracts flavors and preservatives for the beer. The prepared malt is suspended in water mixed with boiled malt adjuncts (other grains, carbohydrates and sugars that provide a source of starch to be converted to sugar). This mash is then incubated at 65 to 70^{o}C for a short time to allow the amylase to break down the starch to glucose. The temperature is raised to 75^{o}C to inactivate the enzymes and then allowed to settle. Insoluble matter sinks to the bottom and serves as a filter as the liquid (now called wort) is taken from the container.

Boiling with Hops – Hops and wort are combined and boiled for 2.5 hours. The liquid is removed and ready for fermentation. Boiling with hops serves several purposes -

- Concentration
- Sterilization, killing many microbes that might spoil the beer
- Further inactivation of enzymes in the mash
- Solubilization of important compounds in the hops and mash. Some of these add to the flavor of the beer while others, especially from the hops, have antiseptic qualities and help preserve the beer.

Fermentation – Fermentation begins by adding the brewers yeast *Saccharomyces carlsbergensis* to the wort. The starter culture is usually obtained from a previous batch of beer and is added at a very high concentration (500 grams per120 liters). Fermentation is at a lower temperature between 3.3 and 14^{o}C for 8 to 14 days. At this time the glucose in wort is converted to ethanol and CO_2. Other compounds in the wort are also fermented to add to the characteristic flavor of beer.

Aging – The fermented wort (green beer) is aged at 0^{o}C for a period of weeks or months depending on the brewer. At this time the yeast settle to the bottom of the vessel, bitter flavors are mellowed and other compounds are formed that enhance flavor.

Finishing – The beer is now prepped for packaging. This can involve filtering, pasteurization, carbonation to 0.45 to 0.52% of CO_2, and clarification. All these processes depend upon the beer being made and each brewery will specialize the fermentation, aging and finishing of their beer. This is often the inspiration for various advertising done by the brewery.

The beer is then put in to containers and distributed to customers. Beer normally has a shelf life of about 6 months and after that, starts to take on undesirable flavors.

Bread

Bread is a simple fermentation of sugar to CO_2 and alcohol. The baker first combines flour, sugar, milk and other ingredients with a microorganism, usually a bread yeast such as *Saccharomyces cerevisiae*, but not always. The ingredients are mixed and then allowed to incubate at 27^{o}C for a few hours. During this time the yeast converts the sugar present to ethanol and CO_2. Most incubations are for less than 4 hours not leaving enough time for the yeast to increase in number. The CO_2 produced causes the bread to rise (leaven) and become porous. The success of leavening is dependent upon the rate of gas production. This can be increased by adding more yeast, more sugar or dough conditioners (various salts that the yeast need). Tweaking a recipe by manipulating these factors can speed CO_2 production, within reasonable limits. Adding too much of anything can either kill the yeast or cause the bread to rise too quickly. The temperature of incubation is another critical consideration. Saccharomyces grows best at 26 to 28^{o}C and deviations from that temperature will usually result in slow or complete lack of leavening. Failure as a baker can normally be attributed to either not adding the exact amounts of ingredients or inappropriate incubation temperatures during leavening.

Yogurt

Yogurt is a product of fermented milk. Lactic acid bacteria are the major microbes in many milk based fermented products. These bacteria are finicky having many growth requirements all of which can fortunately be satisfied by a milk mixture. Lactose in milk is fermented to lactic acid either via the homofermentative or heterofermentative pathway.

Production of yogurt starts by conditioning the milk. The water content of milk is first lowered 25% by vacuum evaporation and 5% milk solids are added. As a final conditioning step, the milk is heated to 86 to 93^{o}C for 30 – 60 minutes. This causes some breakdown of proteins and other molecules and kills contaminating microbes that may compete with the starter culture. After cooling to 45^{o}C a 1:1 mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is added. Fermentation is at 45^{o}C until the desired degree of acidity is reached. This usually occurs in 3 to 5 hours. The finished product may have other ingredients added (such as mold inhibitor or dye) and may be packaged with additional fruit extracts. Yogurt is stored at 0 to 4^{o}C until consumed to prevent spoilage.

Cheese

Cheese is also a milk fermentation, but its production is more complex. Different bacteria come into play and production periods are much longer than yogurt. Despite there being 20 classes and hundreds of varieties of cheeses the initial manufacturing process is surprisingly similar.

Curd Formation – Milk is first pasteurized and then fermented by a starter culture. This is usually a lactic acid bacteria with the specific species in use dependent upon the cheese being produced.

Rennet (a protease) is added to the formation and along with the lactic acid made by the added starter, causes the milk to form curds.

Curd Concentration – Depending upon the cheese being made, the curds may be concentrated in some manner. The goal here is to remove the appropriate amount of whey (liquid left from curd formation). For fresh cheeses (cottage or mozzarella) no concentration is required. For soft cheeses the curds are cut into large cubes and then ripened with a fungus or mold. Hard and semi hard cheeses are cooked and then cut into small pieces to release more whey.

Ripening – Prepared curd is then pressed into molds, salted and ripened for weeks to years. This process is different for each cheese.

The finished product is sold either as a complete mold (a wheel of cheese) or cut into smaller pieces. Most cheeses are stored at refrigerator temperatures.

Foods and Products	**Raw Ingredients**	**Fermenting Organisms**
Dairy Products		
Kefir	Milk	*Streptococcus lactis, Lactobacillus bulgaricus*, Torula sp.
Taette	Milk	*S. lactis* var *taette*
Tarhana	Wheat meal and yogurt	Lactics
Meat and Fish Products		
Country – cured ham	Pork hams	*Aspergillus, Penicllium* spp.
Lebanon bologna	Beef	*Pediococcus cerevisiae*
Fish sauces	Small fish	Halophilic Bacillus spp.
Katsuobushi	Skipjack tuna	*Aspergillus glaucus*
Non Beverage Plant Products		
Cocoa beans	Cacao fruits	*Candida krusei*, Geotrichum spp.
Coffee beans	Coffee cherries	*Erwinia dissolvens*, Saccharomyces spp.
Kimchi	Cabbage and other vegetables	Lactic acid bacteria
Olives	Green Olives	*Leuconostoc mesenteroides, Lactobacillus plantarum*
Pickles	Cucumbers	*Pediococcus cerevisiae, Lactobacillus plantarum*
Beverages		
Bourbon whisky	Corn, rye	*Saccharomyces cerevisiae*
Cider	Apples, other	Saccharomyces spp.
Mezcal	Century plant	Yeasts
Sake	Rice	*Saccharomyces saki*
Vinegar	Cider, wine	Acetobacter spp.
Wine	Grapes, other fruits	*Saccharomyces ellipsoideus* strains

Single Cell Protein (SCP)

Typically refers to sources of mixed proteins extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria (grown on agricultural wastes) used as a substrate for protein – rich foods, in human and animal feeds. SCPs develop when microbes ferment waste materials (including wood, straw, cannery and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).

The SCP needs to be dehydrated to approximately 10% moisture content and / or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels, and de – watering process require equipment that is expensive and not always suitable for small scale operations. It is economically prudent to feed the product locally and shortly after it is produced.

Solid State Fermentation (SSF) for the production of industrial enzymes

Enzymes are the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or the other. Current developments in biotechnology are yielding new applications for enzymes. SSFs hold tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented products may be used directly as enzyme sources.

Biosurfactants

Many microorganisms produce secondary metabolites that have surface – active properties. These compounds are not only useful biosurfactants and bioemulsifiers, but are also indicated as antibiotic and bio – control agents. The majority of microbial surfactants are glycolipids, lipopeptides, and polymeric compounds. However among the most studied are sophorolipids, rhamnolipids and emulsan.

Biosurfactants are usually produced extracellularly or as a part of the cell membrane by yeast, bacteria or filamentous fungi. Different kinds of bacteria have been employed by many researchers in producing biosurfactant using culture media. Most of such bacteria used are isolated from contaminated sites usually containing petroleum hydrocarbon by products and / or industrial

wastes.

Biosurfactants are beginning to acquire a status of potential performance – effective molecules in various fields. Biosurfactants are being studied for their role in enhanced oil recovery and hydrocarbon remediation. Industrial applications of surfactants are classified according to how they are applied. There are surfactants used in detergents and cleaners as auxiliaries for textiles, leather and paper in chemical processes, in cosmetics and pharmaceuticals and in food industry, in agriculture and in others.

Though there are different types of fermentations that are carried out and the outcome product or products of such fermentations differ, fermentation is a process that has many economic advantages, and in many instances are also productions that aid life saving processes, for instance; the manufacture of antibiotics which are life saving molecules.

Encyclopedia

Biopesticides are certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. In most cropping systems, biopesticides should not necessarily be viewed as wholesale replacements for chemical control of plant diseases, but rather as a growing category of efficacious supplements that can be used as rotation agents to retard the onset of resistance to chemical pesticides and improve sustainability. In organic cropping systems, biopesticides can represent valuable tools that further supplement the rich collection of cultural practices that ensure against crop loss to diseases. Biopesticides fall into three major classes:

1. Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds, and other fungi that kill specific insects.

   The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other Bt's are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.
2. Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.
3. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, that interfere with mating, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, EPA has established a special committee to make such decisions.

A major growth area for biopesticides is in the area of seed treatments and soil amendments. Fungicidal and biofungicidal seed treatments are used to control soil borne fungal pathogens that cause seed rots, damping-off, root rot and seedling blights. They can also be used to control internal seed–borne fungal pathogens as well as fungal pathogens that are on the surface of the seed. Many biofungicidal products also show capacities to stimulate plant host defenses and other physiological processes that can make treated crops more resistant to a variety of biotic and abiotic stresses.

Advantages of using Biopesticides

- Biopesticides are usually inherently less toxic than conventional pesticides.
- Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects, and mammals.
- Biopesticides do not leave harmful residues.
- These agents substantially reduce impact on non target species.
- When locally produced may be cheaper than chemical pesticides.
- In the long term may be more effective than chemical pesticides.
- Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high.

Perceived disadvantages of Biopesticides

- To use biopesticides effectively, however, users need to know a great deal about managing pests.
- High specificity, which will require an exact identification of the pest/pathogen and may require multiple pesticides to be used
- Often slow speed of action (thus making them unsuitable if a pest outbreak is an immediate threat to a crop)
- Often variable efficacy due to the influences of various biotic and abiotic factors (since biopesticides are usually living organisms, which bring about pest/pathogen control by multiplying within the target insect pest/pathogen)

Radiation as a means of Sterilization

In the history of medical care the 'concept of asepsis' may be regarded as the most important landmark of advancement. As far back as nearly two centuries ago, microbes were established as the causative agents for disease and infections. This discovery obviously necessitated precautionary measures to keep medical materials free from possible microbial contamination prior to clinical use. This protective step is known as 'Sterilization', which is defined as 'complete' destruction and removal of all forms of contaminating microorganisms from the materials concerned.

Right from the inception of sterilization practices there was an inherent assumption that sterility was 'absolute'. Although the techniques of sterility testing (based upon the testing) of a limited number of items for presence or absence of contaminating microorganisms, may have resulted in 'false positive' tests, the possibility of retest allowed the clearance of products as 'sterile' in most cases. This state of affairs continued as late as the early fifties when statisticians disturbed the microbiologists with the probability theory, according to which sterility becomes a probability.

Sterility testing of products thus became meaningless, because of the small probability of discovering the occurrence of low levels of contamination, however large the sample size in such tests might be. These developments led to the basic recognition that knowledge and control of the process alone could give the greatest assurance of sterility. This radical change in thinking has been at the core of re-shaping the practice and control of sterilization for medical supplies.

Radiation sterilization as we practice it today was introduced, and has since then been found efficient in terms of safety and quality.

Initially scientists utilized the biocidal effects of available physical agents, such as dry and moist heat and certain chemicals (eg., carbolic acid and 70% alcohol) for the sterilization of medical appliances. Interestingly enough, those early sterilizing agents continue to be used, and in the large commercial-scale sterilization operations both heat and toxic ethylene oxide gas are still significant. Both these practices, however, involve the exposure of the bulk medical supplies to the sterilizing agent for a specified duration at controlled temperature, pressure, humidity and vacuum to ensure penetration and uniform effects. The success of the conventional method thus depends on many factors, and an inadequate control of even one of them may lead to failure.

After ionization radiation was used as a means of sterilization, it was necessary to establish many details pertinent to the application of radiation as a sterilization method; including the quantitative relationship between the radiation dose delivered and the microbicidal effect observed, the relative radiosensitivity of different contaminant microorganisms, the influence of environmental conditions prevailing during and after irradiation on the radiation responses (lethality) of the microbes, the quality of radiation including its penetrating powers.

Radiation Sterilization Dose

Since sterilization is a probability function and absolute sterilization (mathematically) is never achieved, it is more accurate to analyze the sterilization process in terms of destruction rate rather than a total destruction time. This rate is designated in mathematical terms as D-value (decimal reduction factor) and is defined as the dose required for the 90% reduction of the population (D_{10}). The following sequences became important:

- Experimental derivation of radiation sensitivity data;
- Development of a dose – survival curve for calculation of the D-value (D_{10}) for the most resistant component(s) of the contaminating population;
- Determination of how the D-values are influenced by the various environmental factors.

It may be generally stated that the choice of sterilizing radiation dose (i.e., the number of D-values needed to yield a certified sterile product) has been based on the calculated D-values of some highly resistant indicator microorganisms as well as the expected concentration (total) of microorganisms in the batch to be sterilized (i.e. derived from the determination of the hygiene standard).

In commercial radiation sterilization of medical products 60Co sources have dominated the scene over electron accelerators.

A radiation sterilization plant using 60Co essentially consists of (i) the radiation source housed in a concrete cell (ii) an automatic conveyer system for carrying the product boxes into the irradiation cell, exposing the boxes to the radiation field for the specified period and taking them out of the cell and (iii) service laboratories for microbiology, dosimetry, etc. The hermitically sealed medical products to be sterilized are packed in standard boxes of certain specified dimension. The boxes are loaded on conveyer belts which carry them at a controlled pre-set speed. The boxes enter the irradiation cell and proceed in a multipass manner (traversing each side of the source four times) and finally return through the irradiation cell ensure the exposure of all the products to a minimum dose of 2.5 megarads. The biological shields and a complete system of safety interlocks protect the operating personnel from hazardous radiation exposure and also protect the products from receiving an overdose or underdose in the event of a mechanical failure.

Factors to be controlled in a reliable sterilization process

Factor	Autoclaving	Gamma Irradiation	Ethylene Oxide Gas
Time	Yes	Yes	Yes
Temperature	Yes	No	Yes
Pressure	Yes	No	Yes
Vacuum	Yes	No	Yes
Concentration (diffusion)	Yes	No	Yes
Wrapping	Yes	No	Yes
Humidity	Yes	No	Yes

Advantages

Radiation as a sterilizing agent offers a number of unique advantages:

(a) Gamma radiation easily reaches all parts of the object to be sterilized due to its high penetrating ability. The items can be pre-packed in hermitically sealed packages, impermeable to microorganisms, before sterilization. Consequently, the sterile shelf – life of these supplies is practically indefinite i.e. up to the point of use.

(b) At the sterilizing dose usually applied, radiation causes no significant rise in temperature. Being a 'cold' process it permits sterilization of heat – sensitive materials, such as plastics. It is certainly the best and often the only method for sterilizing biological tissues and preparations of biological origin.

(c) The chemical reactivity of radiation is relatively low compared with the often highly reactive gases (which may even leave toxic residues hazardous to the patient eg. ethylene oxide gas). The possibility of including a disadvantageous chemical reaction is minimal with radiation.

(d) The sterilizing effect of radiation is instantaneous and simultaneous in the whole of the target and there is no problem similar to convection of heat or diffusion of gas. This also permits the stopping of the effects of radiation at the desired moment. Therefore radiation sterilization is suitable for a continuous, fully automated process, with a single parameter, namely time of exposure, to be controlled. Steam and chemical sterilization apart from being batch processes, require more controls.

The advantages of radiation sterilization has been exploited in many areas including:

- Life sciences; microbiology, medicine,
- Industrial processes; food sterilization, safe packing etc.

However in microbiology the technology has been used beneficially in the sterilization of polystyrene petri plates, which are packed and ready to use, which ensure that the contamination levels are nil, and the quality of the packed plates is such that there are no radiation residues, which may interfere with the cultured microbes, and the features and benefits of such a product is enlisted below:

No interference from residues during culturing

Gamma radiation does not leave any residual toxicity on the plates, which may lethal to the test microorganism.

Ensures complete & 100 % sterility

High penetration power of gamma rays ensures complete sterilization, with no effect on the permeability of the plate material.

Final Pack Sterilization

Ensures that product sterility is retained indefinitely until opened.

Eliminates physical and chemical changes to the plate

It is a 'cold process' with no involvement of heat or chemicals, again which may leave residues that may be toxic to the microbes.

Conclusion

In the modern manufacture of medical supplies, sterilization by radiation is considered the most effective method associated with the greatest safety assurance for public health and quality of the products. From the late fifties till today, the scientific and technological effort expended in the study of radiosterilization practice, including radiation effects on microorganisms, dosimetry and chemical and physical effects on materials, has far exceeded the collective efforts directed to all other sterilization methods. This sterilization method has ushered in a new era of health care and holds even greater potential. The growing interest of the developing Member States to introduce radiation sterilization is a timely one, and should be supported by appropriate regionally co – ordinated programme activities with due emphasis on local conditions.

However in the developing countries as a science like microbiology is gaining momentum, in response to the advancement and the speed at which development in technology is occurring that we too should move ahead with a technology that not only saves us the time for sterilization processes, but has the assurance that when plating is done adequately there will be no contamination which is one of the major reasons why sometimes vital cultures may be lost.

Jules Jean Baptiste Vincent Bordet

Birth: June 13, 1870
Death: April 6, 1961
Nationality: Belgian
Known for: Studies on Humoral Immunity

Jules Bordet was born in Soignies, Belgium, on June 13, 1870. He was educated in Brussels where he graduated as Doctor of Medicine in 1892. In 1894 he went to Paris to work at the Pasteur Institute until 1901 when he returned to Belgium to the Pasteur Institute, Brussels. He has been Director of the Belgian Institute since its inception (honorary since 1940) and Professor of Bacteriology, University of Brussels, since 1907 (honorary since 1935).

Bordet's early studies showed that antimicrobic sera include two active substances, one existing before immunization, known as alexine, and the other a specific antibody created by vaccination: he developed a method of diagnosing microbes by sera. In 1898, he discovered haemolytic sera and showed that the mechanism of their action on foreign blood is similar to that by which an antimicrobic serum acts on microbes and, furthermore, that the reactions of the sera are colloidal in nature. He has contributed much towards the understanding of the formation of coagulin and also anaphylactic poisons. Together with Gengou (in 1906), he cultivated *B. pertussis* and laid foundations of the generally accepted opinion that this organism is the bacterial cause of whooping cough. In addition to his being an acknowledged world authority in many branches of bacteriology, Bordet was considered to be a great exponent and worker on immunology. He was the author of Traité de l'Immunité dans les Maladies Infectieuses (2nd ed., 1939) (Treatise on immunity in infectious diseases) and a great number of medical publications.

Bordet was a permanent member of the Administrative Council of Brussels University, he was President of the First International Congress of Microbiology (Paris, 1930), and Past President of the Premier Council of Hygiene of Belgium, the Scientific Council of the Pasteur Institute of Paris and the Belgian Academy of Medicine. He was Doctor, honoris causa, of the Universities of Cambridge, Paris, Strasbourg, Toulouse, Edinburgh, Nancy, Caen, Montpellier, Cairo, Athens, and Quebec. He was a member of the Belgian Royal Academy, the Royal Society (London), the Royal Society of Edinburgh, the Academy of Medicine (Paris), the National Academy of Sciences (U.S.A.), and many other academies and societies. Bordet gained many awards during his career, including the Grand Cordon de l'Ordre de la Couronne de Belgique (1930), the Grand Cordon de l'Ordre de Léopold (1937), the Grand Croix de la Légion d'Honneur (1938), and public honors of Rumania, Sweden and Luxemburg.

It is Bordet who provided the explanation of the phenomenon. He first of all showed that, provided it is fresh, the cholera immuno-serum always has a bactericidal action, even in a test-tube. Preserved or, better still, heated for a short time to 56°, it loses that property. But he also ascertained that the active property which has disappeared in that way, can be restored by the addition of a small quantity of fresh, non-heated serum taken from a normal animal. The destruction of the vibrios, the bacteriolysis, depends, according to Bordet, on the cooperation of two bodies. One is the thermostable antibody of bacteriolytic immunization formed in the immunized animal and present in its serum; the other exists already in the normal animal; it does not stand up to heating nor to preservation, and does not increase during immunization. Bordet judged the second one to be identical to the slightly bactericidal substances which are found in normal serum and which Buchner has called «alexin». It is also called by the more usual name of «complement». It is therefore definitely proved that bacteriolysis by the immuno-serum derives from the cooperation of a body formed at the time of immunization, the bacteriolytic antibody, and of a substance present in normal serum and not under the influence of immunization, the alexin or the complement. At first, quite naturally, bacteria especially were used for treating animals, since it was desired to immunize them against pathogens and to study their immunization. It was Bordet who first examined the result of the introduction into the organism of foreign cells belonging to different species. He injected guinea-pigs with rabbit blood. In such a case, there formed in the guinea-pig antibodies which, in the presence of alexin or complement, have a destructive action on the red corpuscles of the rabbit, but not on those of other animals. Immediately after the publication of that discovery, similar communications arrived from various quarters.

Bordet's discovery, showing that the introduction of red corpuscles into an animal brings about the formation of a specific antibody, similar in kind to that which forms after the injection of cholera vibrios, was of great importance, especially as it proved that this reaction of the animal organism is a general biological phenomenon. Indeed, analogous results have been obtained since, with a great number of different cells foreign to the test animal. But this discovery of Bordet's was of further fundamental importance as it paved the way to other research work on immunity. The use of bacteria for the study of the properties of antibodies had great drawbacks. Bacteria are living organisms which multiply with extreme rapidity. All the experiments made with living bacteria are, consequently, endangered by the fact that one does not know whether the material to be examined - the bacteria - is constant and, furthermore, to measure their quantity, a great deal of work is often necessary. Those drawbacks, on the other hand, do not exist with red blood corpuscles. The quantity of corpuscles always remains constant, even if the experiments should last many hours. The red coloring matter of the erythrocytes makes them, moreover, a very convenient reagent for research work of that kind because the action of the hemolytic antibody is directly proportional to it and can be directly estimated according to the quantity of coloring matter which, when the red corpuscles are destroyed, is dissolved in the surrounding fluid. This can easily be measured colorimetrically. A very large part of our knowledge about immunity against bacteria and the diseases they provoke is therefore due to the action of hemolytic sera on red corpuscles, and it was only later that attempts were made to find out if, and in what measure, the detected properties apply equally to bacteria and the bacteriolytic sera.

Among other discoveries made by Bordet, a very significant discovery was, however, a discovery of very special importance. In 1900, he ascertained that, with the help of its specific antibody, the substance used to produce immunity fixes the alexin or complement in such a way that, when proportions between the three bodies are favourable, the complement disappears completely from the mixture. The following year, he proved in collaboration with Gengou, that, in all immunizations, there forms specific antibodies which can absorb the complement. In diseases too specific antibodies for the pathogens appear. The fixation of the complement with known microbes can therefore be used to determine the real character of a disease. These were the facts that Wassermann and Bruck took as a basis when they began their experiments to find a specific reaction for the diagnosis of syphilis, experiments which, as we know, were crowned with success. It is true that one of the factors active in the Wassermann test is of a different nature to the analogue of the other fixations of the complement, but it is nevertheless true that this reaction is a true fixation of the complement and that it is based on the previous discoveries of Bordet. These have therefore provided a new weapon to fight syphilis, one of the most terrible plagues of the human race. Bordet's discoveries have thus been of the greatest usefulness for humanity.

In 1899 Bordet married Marthe Levoz. They had one son, Paul, who succeeded his father as Chief of the Pasteur Institute in Brussels and also as Professor of Bacteriology, and two daughters. Jules Bordet died on April 6, 1961.

Enjoy the humour

One sweet young thing arrived at her first football game after the first half. "the score is nothing to nothing," she heard a fan say.

"Oh, good ," she cood to her escort. "then we haven't missed a thing."

If there were four flies on the desk, Eleanor, and I killed one, how many would there be left?" "One," promptly replied Eleanor, "The dead one."

First Student: "Our economics professor talks to himself. Does yours?"

Second Student: "Yes, but he doesn't know it. He thinks we're listening."

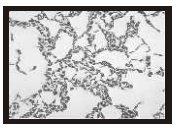
Thoughts to live by

- Business is a combination of war and sport. (Andre Maurois)
- He that would live in peace and at ease must not speak all he knows or all he sees. (Benjamin Franklin)
- Excellence is not a skill. It is an attitude. (Ralph Marston)
- Always bear in mind that your resolution to succeed is more important than any other. (Abraham Lincoln)
- A man begins cutting his wisdom teeth the first time he bites off more than he can chew. (Herb Caen)

Track your brain

1. In fermentation _____ is often an important intermediate.
2. _____ is a fermentation of Barley and hops by yeast.
3. _____ are substances that are obtained from animals, plants and other microbes etc, which are detrimental to certain pests.
4. _____ as a means of sterilization offers several unique advantages as compared to traditional methods.
5. Bordet is known for his work on _____ immunity.
6. Bordet's early studies showed that antimicrobic sera include two active substances, one existing before immunization, known as _____.
7. _____ is a leading cause of *B. cereus* emetic-type food poisoning in the developed countries.
8. In case of suspected *B. cereus* infection _____ tests may be performed to determine the seriousness of the illness.
9. In drugs which are administered orally, _____ may not be a major issue.
10. Some microorganisms are extremely _____ and have complex nutritional requirements.
11. PHMB is an effective _____ and can even be used in neonates and thyroid patients.
12. The incubation _____ can have a major effect on the ability of an organism to grow in a given medium.

Check your Answers on Page 16



Bacillus cereus

Bacillus cereus is an endemic, soil – dwelling, Gram positive, rod – shaped, beta hemolytic bacterium. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals. *B. cereus* bacteria are facultative anaerobes, and like other members of the genus *Bacillus* can produce protective endospores.

B. cereus competes with other microorganisms such as Salmonella and Campylobacter in the gut, so its presence reduces the number of those microorganisms. In food animals such as chickens, rabbits and pigs, some harmless strains of *B. cereus* are used as a probiotic feed additive to reduce Salmonella in the intestines and cecum. This improves the animals' growth as well as food safety for humans who eat their meat.

History

Bacillus cereus has been recognized as an agent of food poisoning since 1955. There are only a few outbreaks a year reported by CDC. Between 1972 and 1986, 52 outbreaks of food – borne diseases associated with *B. cereus* were reported to the CDC (in 2003, there were two), but this is thought to represent only 2% of the total cases which have occurred during these periods. It is not a reportable disease, and usually goes undiagnosed.

Pathogenesis

B. cereus is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting and diarrhea. Generally speaking, Bacillus foodborne illnesses occur due to survival of the bacterial endospores when food is improperly cooked. This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate. Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 and 11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome.

- The diarrheal type is associated with a wide-range of foods, has an 8 to 16.5-hour incubation time and is associated with diarrhea and gastrointestinal pain. Also known as the long-incubation form of *B. cereus* food poisoning, it might be difficult to differentiate from poisoning caused by *Clostridium perfringens*.
- The emetic form is commonly caused by rice that is not cooked for a time and temperature sufficient to kill any spores present, then improperly refrigerated. It can produce a toxin which is not inactivated by later reheating. This form leads to nausea and vomiting 1–5 hours after consumption. It can be difficult to distinguish from other short-term bacterial foodborne pathogens such as *Staphylococcus aureus*.

The diarrhetic syndromes observed in patients is thought to stem from the three toxins Hemolysin BL (HBL), Nonhemolytic Enterotoxin (Nhe) and Cytotoxin K (CytK). These enterotoxins are all produced in the small intestines of the host, thus thwarting the issue of digestion by host endogenous enzymes. The HBL and Nhe toxins are pore-forming toxins closely related to ClyA of *E. coli*, in conformation known as "barrel protein" as they can insert into cellular membranes and cause pores. The effect is loss of cellular membrane potential and eventually cell death. CytK is as well a pore-forming protein more related to other hemolysins.

It was previously thought that the timing of the toxin production might be responsible for the two different types, but in fact the emetic syndrome is caused by a toxin called cereulide that is found only in emetic strains and is not part of the "standard toolbox" of *B. cereus*.

B. cereus is also known to cause people to have skin infections that can be quite lengthy, difficult to eradicate, and damaging, though less aggressive than the more notorious necrotizing fasciitis. *B. cereus* can also cause keratitis.

Symptoms

B. cereus causes two types of food – borne illnesses. One type is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6 hours. It resembles *Staphylococcus aureus* (staph) food poisoning in its symptoms and incubation period. This is the 'short incubation' or emetic form of the disease.

The second type is manifested primarily by abdominal cramps and diarrhea following an incubation period of 8 to 16 hours. Diarrhea may be a small volume or profuse and watery. This type is referred to as the 'long – incubation' or diarrheal form of the disease, and it resembles food poisoning caused by *Clostridium perfringens*. In either type, the illness usually lasts less than 24 hours after onset. In a few patients symptoms may last longer.

The short – incubation form is caused by a preformed, heat – stable emetic toxin, ETE. The mechanism and site of action of this toxin are unknown, although the small molecule forms ion channels and holes in membranes. The long incubation form of illness is mediated by the heat – labile diarrheagenic enterotoxin Nhe and / or hemolytic enterotoxin HBL, which cause intestinal fluid secretion, probably by several mechanisms, including pore formation and activation of adenylate cyclase enzymes.

Epidemiology

B. cereus food poisoning occurs year-round and is without any particular geographic distribution. The short-incubation form is most often associated with rice dishes that have been cooked and then held at warm temperatures for several hours. It is often associated with Mexican and Chinese restaurants, but in one reported outbreak, macaroni and cheese made from powdered milk turned out to be the source of the bacterium.

Long-incubation *B. cereus* food poisoning is frequently associated with meat or vegetable-containing foods after cooking. The bacterium has been isolated from 50% of dried beans and cereals and from 25% of dried foods such as spices, seasoning mixes and potatoes. One outbreak of the long-incubation form was traced to a "meals-on-wheels" program in which food was held above room temperature for a prolonged period before delivery to consumers.

The short-incubation or emetic form of the disease is diagnosed by the isolation of *B. cereus* from the incriminated food. The long-incubation or diarrheal form is diagnosed by isolation of the

organism from stool and food. Isolation from stools alone is not sufficient because 14% of healthy adults have been reported to have transient gastrointestinal colonization with *B. cereus*. Because *B. cereus* gastroenteritis is generally a benign, self-limited illness, antimicrobial agents are of no value in management. Since the bacteria grow best at temperatures ranging from 40 to 140°F, infection may be prevented if cold food is refrigerated and if hot food is held at greater than 140°F before serving.

Fried rice is a leading cause of *B. cereus* emetic-type food poisoning in the developed countries. *B. cereus* is frequently present in uncooked rice, and heat-resistant spores may survive cooking. If cooked rice is subsequently held at room temperature, vegetative forms multiply, and heat-stable toxin is produced that can survive brief heating, such as stir frying. In certain outbreaks, vegetative forms of the organism probably multiplied at the restaurant and the day care centers while the rice was held at room temperature.

The day care staff and restaurant food handlers in such situations may have been unaware that cooked rice was a potentially hazardous food. Therefore there is a need to educate food handlers about basic practices for safe food handling.

Diagnosis and Treatment

When a gastrointestinal problem is suspected, a physical examination is conducted which includes measurements of blood pressure, pulse, breathing rate and temperature in order to check outward signs and symptoms of the illness. They will assess how dehydrated the patient is and examine the abdominal area to make sure the illness is not serious.

A sample of stool is taken and tested for blood and mucus. In some cases, a sample of stool or vomit can be sent to the laboratory for further testing to find out which toxin caused the disease.

A urine sample helps assess how dehydrated the patient is and may indicate possible kidney damage.

Blood tests may be performed to determine the seriousness of the illness. An X-ray of the abdomen or a CT scan may be taken if the doctor suspects your symptoms may be caused by another illness.

Self Care at Home

- Short episodes of vomiting and small amounts of diarrhea lasting less than 24 hours can usually be cared for at home.
- Do not eat solid food while nauseous or vomiting but drink plenty of fluids.
- Small, frequent sips of clear liquids (those you can see through) are the best way to stay hydrated.
- Avoid alcoholic, caffeinated, or sugary drinks. Over – the – counter rehydration products made for children, if available are a good choice.

After successfully tolerating fluids, eating should begin slowly, when nausea and vomiting have stopped. Plain foods that are easy on the stomach should be started in small amounts. Consider eating rice, wheat, breads, potatoes, low sugar cereals, lean meats, and chicken (not fried) to start. Milk can be given safely, although some people may experience additional stomach upset due to lactose intolerance.

Medical Treatment

The main treatment for food poisoning is putting fluid back in the body (rehydration) through an IV and drinking fluids. The patient may need to be admitted to the hospital. This depends on the severity of the dehydration, response to therapy, and ability to drink fluid without vomiting. Children, in particular, may need close observation.

- Anti – vomiting and diarrhea medications may be given.
- The doctor may also treat any fever to make the patient more comfortable.
- Antibiotics are rarely needed for food poisoning. In some cases, antibiotics worsen the condition. Only a few specific causes of food poisoning are improved by using these medications.

Prevention

Safe steps in food handling, cooking, and storage are essential to avoiding food-borne illness. Bacteria cannot be seen, smelled, or tasted, which may be on any food.

Follow the CDC food safety guidelines to keep contaminants away.

- Safe shopping
  (1) Buy cold foods last during your shopping trip. Get them home fast. (2) Never choose torn or leaking packages. (3) Do not buy foods past their "sell-by" or expiration dates. (4) Keep raw meat and poultry separate from other foods.
- Safe storage of foods
  (1) Keep it safe; refrigerate. (2) Unload perishable foods first and immediately refrigerate them. Place raw meat, poultry, or fish in the coldest section of your refrigerator. (3) Check the temperature of your appliances. To slow bacterial growth, the refrigerator should be at 40°F, the freezer at 0°F. (4) Cook or freeze fresh poultry, fish, ground meats, and variety meats within two days.
- Safe food preparation
  (1) Keep everything clean! (2) Wash hands before and after handling raw meat and poultry. (3) Sanitize cutting boards often in a solution of one teaspoon chlorine bleach in one quart of water. (4) Do not cross-contaminate. Keep raw meat, poultry, fish, and their juices away from other food. After cutting raw meats, wash hands, cutting board, knife, and counter tops with hot, soapy water. (5) Marinate meat and poultry in a covered dish in the refrigerator. Discard any uncooked/unused marinade.
- Thawing food safely
  (1) Refrigerator: Allows slow, safe thawing. Make sure thawing juices do not drip on other foods. (2) Cold water: For faster thawing, place food in a leak-proof plastic bag and submerge in cold tap water. (3) Microwave: Cook meat and poultry immediately after microwave thawing.
- Safe cooking
  (1) Use a meat thermometer. (2) Cook ground meats to 160°F; ground poultry to 165°F. Beef, veal, and lamb steaks, roasts and chops may be cooked to 145°F; all cuts of fresh pork, 160°F. Whole poultry should reach 180°F in the thigh; breasts 170°F. (3) Keep hot foods hot and cold foods cold. (4) Never leave food out more than two hours (or more than one hour in temperatures above 90°F). (5) Bacteria that cause food poisoning grow rapidly at room temperature. (6)Use cooked leftovers within four days.

Povidone-iodine as an Antiseptic? Think Again

There are several compounds that are used for antisepsis and disinfection and these include iodine and its compounds. Though less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they're often associated with irritation and excessive staining. In addition aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I_2) being primarily responsible for antimicrobial efficacy. These problems were however overcome by the development of iodophors ('iodine-carriers' or 'iodine-releasing agents'); the most widely used are povidone iodine and poloxamer iodine in both antiseptics and disinfectants. Iodophor antiseptic solutions are currently used in health care for many purposes, including skin antisepsis before surgery, irrigation of wounds and ulcers, and in many instances, incorrectly for disinfection of patient-care items.

Similar to chlorine, the antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins, nucleotides, and fatty acids, which culminates in cell death. Less is known about the antimicrobial action of iodine, but non lipid viruses and parvoviruses are less sensitive than lipid enveloped viruses. Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds.

Unfortunately, iodophors (povidone-iodine)

(1) Have a minimal residual effect. (2) Get deactivated in the presence of organic matter and body fluids. (3) Not recommended in neonates, particularly pre-term infants. (4) Not recommended for thyroid patients. (5) May be toxic to tissues. (6) Allergy/ hypersensitivity is possible.

Clinically adverse effects from use of contaminated antiseptic iodophor solutions in patient care have also been documented, Poloxamer-iodine and povidone-iodine are the most commonly used iodophor preparations in hospitals; both preparations have now been demonstrated to be vulnerable to intrinsic contamination. According to the Centers for Disease Control and Prevention (CDC), USA, *P. aeruginosa* is the fourth most commonly isolated nosocomial pathogen, accounting for 10.1% of all hospital-acquired infections. Several different epidemiological studies indicate that antiseptic resistance is increasing in clinical isolates. Combined with the ability to form biofilms, *Pseudomonas aeruginosa* is able to survive in a variety of unexpected places. For example, in production areas of pharmaceutical industries, antiseptics such as povidone iodine, quaternary ammonium compounds, bottled mineral water and a simple carbon source, such as soap residue or cap liner-adhesives.

In 1980, a cluster of pseudobacteremias (caused by *P. cepacia*) in seven northeastern U.S. hospitals was associated with a contaminated povidone-iodine solution used to disinfect the tops of blood-culture bottles before inoculation. In 1982, a cluster of peritonitis (caused by *P. aeruginosa*) cases in peritoneal dialysis patients was associated with a contaminated poloxamer-iodine solution being used as a peritoneal catheter disinfectant in a hospital. Data from the 1980 and 1982 outbreaks suggested that *P. cepacia* and *P. aeruginosa*, organisms commonly found in water, could colonize water distribution pipes or filters in plants that manufacture iodine solutions. Subsequent laboratory studies revealed that, once affixed to the inner surface of polyvinylchloride distribution pipes and pipes of other compositions, *P. cepacia* and *P. aeruginosa* could be protected from the bactericidal effect of the iodophor solution, probably by a glycocalyx film. In addition, studies conducted by Corson *et al.*, demonstrated growth of common skin microorganisms (e.g., *Staphylococcus epidermidis*, diphtheroids) from the umbilical area even after skin preparation with povidone-iodine and ethyl alcohol. Similar organisms were recovered in some instances from the pelvic serosal surfaces or from the laparoscopic telescopes, suggesting that the microorganisms probably were carried from the skin into the peritoneal cavity.

In terms of antisepsis, modern disinfectants, such as Poly(hexamethylenebiguanide)hydrochloride (PHMB) is an excellent choice. PHMB, a polymeric biguanide, is a broad spectrum cationic surface active antimicrobial agent. In accordance with CDC guidelines - new disinfection methods should include a persistent antimicrobial that can be applied to inanimate and animate objects, it is also a multipurpose antimicrobial agent that can be used for skin, surface and instrument disinfection.

Benefits of PHMB:

- **Chemically stable & non volatile.** (1) PHMB has very low surface activity, having a surface tension essentially identical to water, & consequently can be readily water rinsed from surfaces & do not have residual streaks or tackiness. (2) Odorless, non foaming, clear & colorless. (3) Easily handled & applied. (4) Effective & stable over a wide pH range (4-10).
- **Unique biguanide chemistry.** (1) Novel non specific mode of action. (2) No known evidence of development of organism resistance.
- **Broad spectrum of activity.** (1) High activity against Pseudomonas, MRSA, VRE, food borne pathogenic organism, viruses & so on. (2) Retains activity in presence of organic matter.
- **Safe antiseptic.** (1) Not cytotoxic to human cells. (2) No skin sensitization/irritation. (3) Can be used for thyroid patients.

How does PHMB work?

(1) A rapid electrostatic attraction occurs between the positively charged PHMB and the negatively charged bacterial cell surface. (2) The PHMB fights for the negative sites on the cell wall, thereby displacing metallic cations essential to the integrity of the cell outer membrane. (3) PHMB gets attracted towards the cytoplasmic membrane (composed of acidic phospholipids - essential for expanding and preventing collapse of the two bi-layers of the membrane; functional proteins embedded in the membrane also depend on the boundary phospholipids for activity) and destabilizes it. (4) The phospholipids of the membrane form aggregates of PHMB surrounding essential proteins leading to loss of protein function. At this point, an extensive disruption of the membrane with leakage of macromolecular components from the cell, such as nucleotides takes place. This eventually leads to the precipitation of the majority of cell contents and death of the bacterial cell.

Why PHMB is better than traditionally used povidone iodine?

(1) Unlike povidone iodine, PHMB is not inactivated in the presence of organic matter (2) Unlike povidone iodine, PHMB is not cytotoxic. (3) Unlike povidone iodine, PHMB can be used for thyroid patients. (4) Unlike povidone iodine, PHMB is resistance free. (5) PHMB is not affected by sunlight, water, temperature and pH fluctuations. This stability makes PHMB a better antimicrobial agent. (6) PHMB has Low acute toxicity via dermal & oral route. (7) Low skin & eye irritation potential at in-use concentration. (8) Low toxicity following long term exposure. (9) Non teratogenic & shows no reproductive effects when studied over two generations. (10) Non genotoxic in range of studies.

Validation of Microbiological Test Methods

Validation of Microbiological Test Methods has become a formalized methodology within the pharmaceutical, biotechnology, and medical device industries during the past five years as methods have become more company specific and have drifted away from the standard United Stated Pharmacopoeia (USP) assays. Validation of Microbiological Test Methods is designed to assist in the validation of test methods using a step–by–step procedure for manual and compendial methods, automated microbiological systems and rapid methods. Various interactive exercises have been used to assist in familiarization of this process. Other areas include sterile and non sterile product and process requirements, cleaning validation, water, air and surface monitoring and bioburden analysis.

The variety of microbiological tests makes it difficult, if not impossible, to prescribe a single, comprehensive method for validating all types of tests. By their very nature, microbiological tests possess properties that make them different from chemical tests. Consequently, the well-known procedures for validating chemical tests are not appropriate for many microbiological tests. Yet, it is necessary to validate microbiological tests if they are to be useful for controlling the quality of drug products and devices. Test-method validation provides assurance that a method is suitable for its intended use.

Some tests, such as bioburden or viral titer tests, are quantitative in nature while other tests, such as those for the presence of objectionable organisms, are qualitative. As with chemical tests, these differences necessitate different validation approaches. The purpose of a test may also change the procedures for running and validating it. As an example, consider a drug that will be orally administered. Normally, sterility is not a major issue, and the specification allows for a considerable number of organisms. However, if the drug will be administered to immunocompromised cancer or AIDS patients, the bioburden level must be reduced considerably, increasing the test sensitivity required in the validation study.

The nature of the test material itself changes how a test is run and the validation protocol. Consequently, testing for objectionable organisms is different when testing a diuretic for hypertension or an antibiotic for treating pneumonia. Also, a procedure that works perfectly well for checking the bioburden of granulated sugars may fail with sodium chloride. These differences make full coverage of the topic impossible within the context of this primer.

Note also that certain microbiological tests are already associated with well defined validation procedures. For example, the endotoxin test and USP bacterial enumeration tests have clearly defined validation procedures. In addition, individual countries may have specific requirements that modify or change standard procedures. If a test is associated with a compendial or regulatory validation procedure, workers are advised to follow that procedure unless there are clear reasons for not doing so. In such cases, the reasons should be documented and filed with the test procedure.

Media

The suitability of the medium used for cultivating organisms or cells obviously can have a major impact on the test results. Some organisms are extremely fastidious and require a precisely defined medium with several complex nutrients, while others grow in the presence of inorganic salt mixtures and simple carbon sources. It is commonly argued that delicate, fastidious organisms cannot survive manufacturing processes and should not be of concern, but organisms as delicate and fastidious as mycoplasmas can appear in final preparations of biologics.

In addition to the nutrient composition of the media, more general factors such as pH and ionic strength must be validated. While it is commonly believed that media in the range of pH 6.0 – 8.0 are suitable for sterility and bioburden studies, individual organisms may require a more restricted range. The same holds true for ionic strengths and osmolalities outside of the human physiological range. Shifting the pH range from 6.0 – 7.0 to 7.0– 8.0 and raising the ionic strength to 300 mOsm may select for a different set of organisms than those that would be present in the lower pH range at 150 mOsm.

Most validation schemes require the use of five or more "indicator organisms" to demonstrate the medium's ability to support growth. In addition to aerobic bacteria, anaerobic organisms, yeasts, and molds are usually included. This is an important step since a finding of "no growth detected" is meaningless if the medium was incapable of growing any organisms. This leads to two important points.

First, the indicator organisms are supposedly representative of the types of organisms that will be encountered during the testing, but this is not necessarily true. The indicator organisms are a subset of organisms that are known to grow on properly prepared media, but the organisms contaminating a manufacturing process may not belong to that subset. As a result the quality control laboratory may repeatedly face what appears to be a microbial contamination event despite monitoring cultures that show no growth. It is very important to know what organisms are normally present in the working environment and to include these environmental isolates in a validation program. There is little value in proving that a medium will support the growth of indicator organisms if the environment is full of organisms with very different cultivation requirements.

The second issue involves media handling. The qualification or validation study may require autoclaving the medium and then pouring culture plates as the autoclaved material cools. In laboratories with a low testing load, the excess material is often poured into large tubes or culture flasks to cool and solidify and then stored for future use, usually in a refrigerator. However, when future testing is done, the second heating of the medium may not be captured in the qualification or validation check and may not even be mentioned in the test procedure. If the agar is melted under gentle conditions and quickly poured, there may be no problem, but in some cases, technicians have placed the flasks in microwave ovens to heat the medium while taking a short break. With a powerful microwave oven it is easy to boil the medium for an unknown period of time. This can destroy nutrients or produce toxic or inhibitory substances. Consequently, in laboratories where this second heating is a common practice, this procedure must be captured in the validation and described exactly in the test procedures.

When preparing the validation protocol, the analyst should specify the recovery level expected for each of the indicator organisms.

Generally, recovery of at least 80% of the inoculum or control is desirable. Recovery of less than 50% is usually unacceptable and should raise questions about the presence of inhibitory substances, especially when the testing is taking place in the presence of a raw material or product intermediate. It may be necessary to introduce — and validate the performance of — an agent that inactivates the inhibitor. It is important to set the specifications before the study is conducted and to hold to these specifications. If specifications are not pre-set and the test system cannot meet general acceptance specifications, it is very easy to set "acceptable" specifications that would otherwise have been unacceptable. The other problem is the "specification creep" that occurs when a recovery of 78% is found and the specification is 80%. A quality assurance or quality control worker who allows the 78% to pass will soon face the expectation that 75% should pass because it is "only slightly different from the other one." Over the course of a few years, an 80% specification can gradually turn into a 70%, then 65%, specification.

Environment

The incubation temperature can have a major effect on the ability of an organism to grow in a given medium. It is well known that yeasts and molds require a different incubation temperature than bacteria in a sterility test. Similarly, cells in tissue culture are often extremely sensitive to small changes in temperature, not only for their growth but also in their susceptibility to being infected or lysed by viruses. The analyst may need to develop temperature curves to justify the incubation temperatures used for the test. It is also important to verify the incubator's ability to maintain the set temperature within the specified range. If a four-degree temperature variation can cause a significant change in the test results, the incubator's ability to hold a ±1° C range at all internal locations is critical. This may not be covered in a validation study, but it should be included in the incubator's qualification studies.

In addition to the usual range from 20 – 40° C, it may be necessary to demonstrate the ability to grow organisms at extreme temperatures. If it is necessary to monitor the presence of microbes in a hot or cold room, it will be necessary to demonstrate an ability to cultivate thermophiles or psychrophiles in addition to organisms that grow under more normal conditions. While the significance of these extremophiles may be open to question, their presence and the possibility that they may leave residues such as endotoxins must be considered.

The atmosphere in which the test system is immersed can have a major effect. Anaerobic organisms cannot grow in the presence of oxygen, and tissue cultures may require the presence of 5% CO_2 to grow well. Certain facultative organisms will adjust their metabolic paths to cope with reduced levels of oxygen. This, in turn, can affect their growth rates. When media for general purposes, such as sterility tests, are being considered, it is normal to include one medium that provides anaerobic conditions. The detection of anaerobes is important as they include toxin-producing and other pathogenic bacteria.

Quantitative Issues

One of the problems with quantitative microbiological tests is that as microbe counts become smaller, straight-forward linear behavior is less common than that which follows the Poisson distribution. This is because random distribution is not even distribution. Most quantitative tests for microorganisms require the plating of dilute liquid samples, and it is normal to prepare samples to ensure the dispersion of microbes and a random distribution of bacteria or viruses. When concentrations are high, the lack of even distribution is not a problem; simple linear averaging methods can compensate for the uneven distribution. Problems arise with smaller numbers of microbes.

Consider an example where there are exactly 100,000 organisms per mL. If 0.1 mL is taken and mixed with 0.9 mL of a diluent, it is highly unlikely that the new suspension will contain exactly 10,000 organisms; it would not be surprising to have anywhere from 9,800 – 10,200 organisms. Back-calculating the result produces a range from 98,000 – 102,000 organisms in the original sample, and, if there were enough replicates, the results could be averaged to obtain a number indistinguishable from 100,000. This is the result that would be expected based on linear thinking.

However, if there were only 10 organisms per mL, it is quite possible that a 0.1 mL aliquot would not contain any organisms at all. In fact, in this situation about one third of the aliquots will not contain a single organism. This could lead to the conclusion, on averaging, that the sample only contained 6.7 organisms per mL, which is a significant deviation from the true value.

A transition occurred from a high density that produces a fairly smooth, homogeneous distribution of organisms to a low density that results in organisms that are distributed with significant distances between them. Under these conditions, the suspension behaves according to the Poisson distribution and assumptions related to a normal distribution no longer hold. The Poisson distribution is an exponential function. The problem is that parameters such as the standard deviations may be logarithmic in nature, and when attempts are made to make these numbers "real" by taking the antilogarithms, the results may actually have no "real" meaning. This can cause great difficulties when attempting to validate quantitative microbial test procedures.

When it is necessary to deal with the Poisson distribution, it is wise to consult a statistician who is versed in the use of this distribution. It appears that the transition to the Poisson distribution occurs when approximately 100 colonies or plaques are counted. This is unfortunate because at this level many analysts will declare a colony or plaque count to be "too numerous to count" (TNTC) to avoid the tedium of these measurements. Therefore, most colony or plaque counting procedures actually operate under the Poisson distribution and calculations based on the normal distribution will be incorrect.

Revalidation

The frequency of revalidation is a contentious question. There are many tests, such as the growth promotion test on culture media, that are essentially self-validating and are run frequently. It could be argued that if performance parameters (for example, percent recovery of indicator organisms) are monitored via control charting and no significant changes are seen, revalidation is unnecessary. However, control charting usually does not measure all the parameters included in validation studies. Consequently, it is wise to revalidate tests after any major change in constituents or procedures; in fact, revalidation may be needed to justify the changes. Changes in suppliers (especially of media components) and changes in the composition of test samples have resulted in major changes in microbiological tests. Finally, it is probably wise to re - validate procedures approximately every second year to protect against unseen or unreported changes. A media supplier may change its own suppliers or change its processing procedures without notifying customers. The supplier may have no idea of the impact these changes could have on the end use of their product. In addition, personnel changes in the laboratory and the maturing of analysts' techniques can also have an effect.

BioShields Presents Nusept

Composition - 1% w/v Poly (hexamethylene biguanide) hydrochloride, Perfume, Fast green FCF as color.

Description: NUSEPT™ is a new generation, powerful, non stinging, safe, highly effective and resistance-free microbicidal antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility is affected by the induction or hyper expression of multi-drug efflux pumps, neither there have been any reports of acquired resistance towards this agent.

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

CONTACT TIME :1 min (undiluted & 10% v/v solution), 5 min (5% v/v solution), 10 min (2.5% v/v solution).

APPLICATIONS :

Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.

Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE DIRECTIONS :

Surgical, postoperative, non surgical dressings	–	Use undiluted
Pre & post surgery, skin cleaning & disinfection	–	Use undiluted
Surgical/Sitz bath	–	Add 50 ml of NUSEPT™ in 1L of water & use
Antisepsis during minor incisions, catheterization,	–	Use undiluted scopy, first aid, bites, cuts stings etc
Midwifery, nursery & sickroom	–	Use undiluted
General surface disinfection	–	Add 100 ml of NUSEPT™ in 1L of water and gently mop the floor or surfaces

Track your brain

1. Pyruvate
2. Beer
3. Biopesticides
4. Radiation
5. Humoral
6. Alexine
7. Fried rice
8. Blood
9. Sterility
10. Fastidious
11. Antiseptic
12. Temperature

Microxpresss offers a whole range of :

Gamma irradiated ready to use sterile plates

Cat. No.	Description	Quantity	PackSize
MXPPG 9001	Gamma plates, sterile, 90 mm X 15 mm, disposable plate, individually packed.	450	(1X450 plates)
MXPPG 9012	Gamma plates, sterile, 90 mm X 15 mm, disposable plate.	552	(12 X 46)
MXPPG 5511	Kontact plate, sterile, 55 mm X 15 mm, disposable scored plate for surface monitoring.	1122	(11 X 102)

Ready prepared media for cultivation of *Bacillus cereus*

Bacillus cereus Agar Base

A selective medium for the isolation and enumeration of *Bacillus cereus.*

Gelatin Broth

A medium for isolation and enumeration of *Bacillus cereus*.

Dextrose Peptone Medium

A general purpose medium for the cultivation of a wide variety of microorganisms.

Heart Infusion Agar

A general purpose medium used in the cultivation of a wide variety of microorganisms from a variety of clinical and non clinical specimen.

Yeast Extract Agar

A highly nutritious medium for the cultivation of a wide variety of microorganisms.

Soyabean casein Digest Medium

A medium for the isolation and cultivation of a wide variety of microorganisms

Ready prepared media for cultivation of microorganisms in brewing and Fermentation

Raka Ray Medium (lactic Acid Bacteria selective medium)

A selective medium for the isolation of Lactic Acid Bacteria in beer and brewing processes.

Universal Beer Agar (UB Agar)

A medium for cultivating of microorganisms related to brewing industry.

Highlights of the coming issue

Printed and published by D.G. Tripathi, Edited by Gracinda Mesquita for and on behalf of Tulip Diagnostics (P) Ltd., Tulip House, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex, Post Office Goa, 403 202, India. Fax- 0832 2458544, E-mail: sales@tulipgroup.com, Website- www.tulipgroup.com.

Bimonthly Journal Published Exclusively For Tulip Group Customers