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A221: Microbiology

Problem 3: Check it out! Part I

WORKSHEET

Question 1

Micky noticed that her grandmother boiled the pot of left-over curry before going to bed.

a) Why do you think Micky's grandmother boiled the pot of left-over curry?
Micky's grandmother boiled the pot of leftover curry to kill the bacteria and eat hot curry.

b) How has the boiling process helped in the answer you have provided in part a?
The boiling process helped in killing bacteria and heat up the curry.

c) Do you think preparation of growth media for microorganisms can be done by boiling the media, just like the curry?
Yes, preparation of growth media for microorganisms can be done by boiling the media because different microorganisms can grow at different temperatures. Some microorganisms' optimal growth could be the boiling point of the curry.

Question 2

The table below shows the effective temperature and time required for two modes of sterilization.

	Temperature	**Time to sterilize**
Steam	121°C	15 min
Hot air in oven	160°C	120 min

a) What does sterilization mean?
Sterilization means destroys or removes all viable microorganisms.

b) Why is sterilization carried out at such a high temperature?

Sterilization is carried out at high temperatures because most microorganisms cannot survive in environments of high temperatures.

c) From the above table, it seemed that sterilization by the hot air in oven requires much longer time than steam. Why is this so?
Due to dry heat in the oven, it allows the bacteria to quickly form endospores for it to be heat resistant and survive in high temperatures. However, steam will 'trick' bacteria to think that the environment is favourable so it will divide and the heat will then kill the bacteria. Thus, sterilization by the hot air in oven requires much longer time than steam.

d) Most of the materials in laboratory are autoclaved before being used in experiments.

What is the normal autoclave settings (temperature, pressure and time) used in laboratories?
The normal autoclave settings are 121 °C (249 °F) for around 15–20 minutes and 15 to 20 kPA / psi.

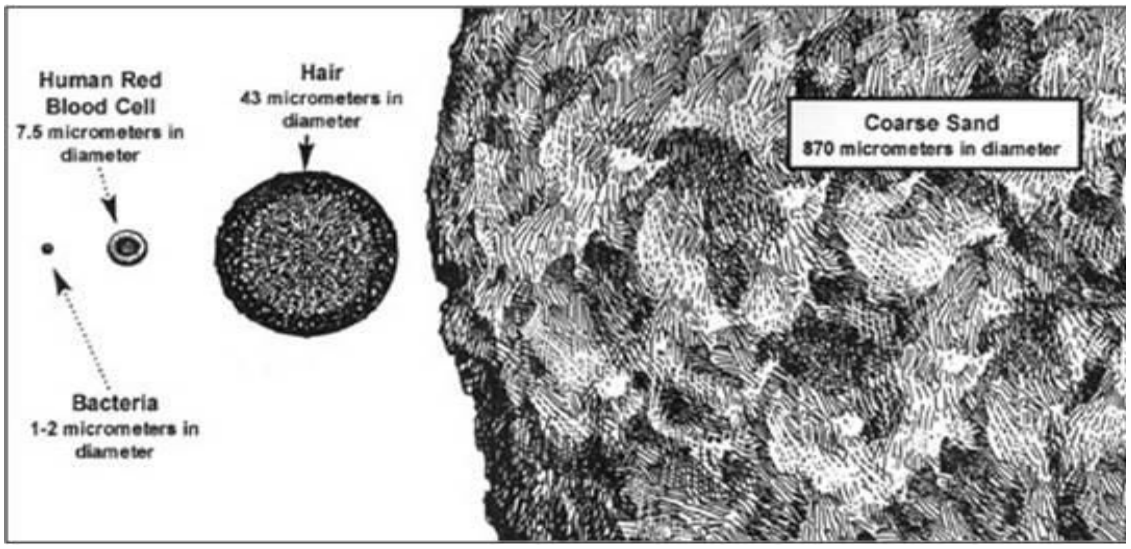
e) Why is it necessary to create pressure in the autoclave?
High pressure enables the steam to reach high temperatures, increasing its heat content and killing power.

f) What do you think will happen if egg white is autoclaved?
The egg white will denature, coagulate and be cooked. (become solid)

g) Similarly, if an enzyme solution is required to be sterilized for experimental purposes, would autoclaving be an ideal method to achieve this purpose? Why or why not?
Autoclaving would not be an ideal method to sterilize the enzyme solution. This is because autoclaving involves raising the temperature of the item that is to be sterilized, thus the enzyme solution might denature if autoclaved (enzymes will lose its function when the temperature is too high).

Question 3

The diagram below shows a comparison of the size of an average bacterium to red blood cell, hair and sand.



a) The table below shows 3 different types of filter with different pore sizes. Complete the table to indicate the material(s) that will pass through or be retained when a mixture of coarse sand, hair, human red blood cell and bacteria is sieved through the respective filters.

	Pore size	**Material(s) that will be retained**	**Material(s) that will pass through**
Filter 1 	1 mm	Nothing	Coarse sand Hair Human red blood cell Bacteria
Filter 2 	0.1mm	Coarse sand	Hair Human red blood cell Bacteria

Filter 3 	0.2µm	Coarse sand Hair Human red blood cell Bacteria	Nothing

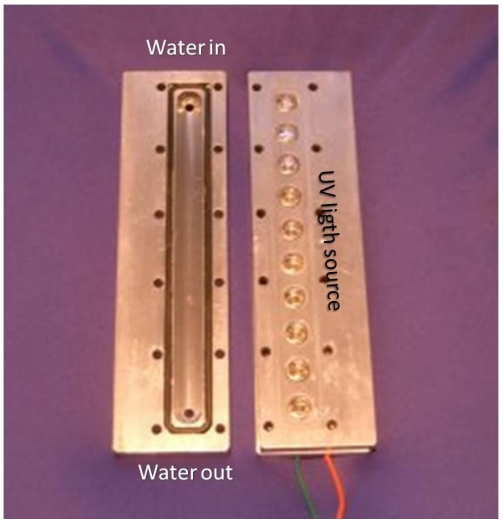
b) Which of the above filter is useful for laboratory purposes? Why?
Filter 3 is useful for laboratory purposes because it is able to prevent contamination by filtering out any small unwanted particles.

c) When will such filters be utilized in laboratories?
Such filters will be utilized in laboratories for separation and filtration.

d) List some potential limitations of the filter you had listed in part (b).
Some potential limitations are that some substances that are too small will still pass through.

Question 4

The figure below depicts a water purifier unit that makes use of ultraviolet light (UV) to eliminate bacteria in drinking water. The UV light source will be place directly onto of the stream of passing water to purify the water.



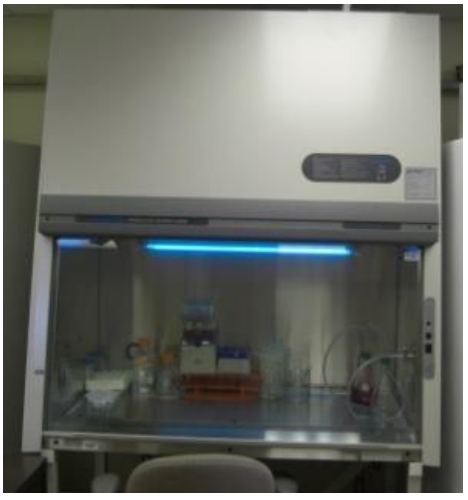
a) How does UV radiation result in the elimination of bacteria?
UV radiation damages the DNA of the bacteria causing thymine dimers to form hence resulting in the elimination of bacteria.

b) Examine the water purifier unit (watch video below), you can see that only a narrow stream of water is allowed to pass through the UV light source. Why do you think this is so?

UV Water Filter
Purification Systems I

When only a narrow stream of water passed through the UV light source at a time, the UV light will be able to kill the microorganisms better as compared to having a large stream of water. In a narrow stream of water, there would be lesser microorganisms present, allowing the UV light to kill them easily.

c) Besides being utilized in purification of water, UV has also been commonly used for the disinfection of biosafety cabinet as shown in the figure below. Biosafety cabinet provides an aseptic environment for work to be carried out and also protects the user from biological hazards.



In order to maintain an aseptic environment in the biosafety cabinet, the materials have to be swabbed with 70% ethanol to remove contaminants of the surface of the materials.

i. Why is this considered as a method of disinfection and not sterilization?
Disinfecting helps to eliminate or reduce vegetative microorganisms from objects or surfaces while sterilization is the process of completely killing all microorganisms, for example in a solution.
This method is considered as disinfection because it only remove contaminants on the surface of the materials.

ii. What is alcohol's mechanism of action to remove contaminating microorganisms?
The alcohol's mechanism of action to remove contaminating microorganisms are protein denaturation and dissolving the lipid membrane (disruption of of the bacterial membrane) in order to kill the microorganisms.

iii. Will the mechanism you have suggested in Part ii be affected if 100% ethanol is being used instead?

Yes, the mechanism will be affected if 100% ethanol is being used instead. Ethanol of 70% would destroy the cell membrane of microorganisms by dissolving their lipids and denaturing their proteins.

However, if we use 100% ethanol instead, it would evaporate faster and may not be as effective. Moreover, microorganisms are denatured more readily in the presence of water. Water helps to reduce rapid precipitation of outer proteins, which would allow more ethanol to penetrate deeper into the microorganism.

d) Liquid bacterial cultures in the laboratory are considered to be biohazards and have to be disposed of safely. Autoclaving is one way of ensuring sterilization while the other way is to ensure disinfection where all the pathogenic bacteria in a culture are killed with sufficient exposure to 10% bleach/water made fresh daily with bleach (e.g. Clorox) for 30 minutes. What is the mechanism of action that makes bleach an efficient disinfectant in the lab?

The mechanism of action that makes bleach an efficient disinfectant in the lab is that bleach causes unfolding of proteins in bacteria.

Going further (Optional):

Let us now explore some sterilization/ disinfection methods that are commonly employed in the industries with the help of the following links.

- http://www.chemengonline.com/large-scale-fermentation-systems-hygienic-design-principles/?printmode=1
- http://www.gammapak.com/en/farmasotik-kozmetik.html

Industries	Applications	Methods
Food	Cleaning up of fermenter	• Low pressure steam • •
Biopharmaceutical	Sterile medical products	• Steam heat sterilization • Filtration sterilization

		• Gamma ray sterilization (irradiation technology) • Chemical sterilization (Eto gas and formaldehyde)
Laboratories handling highly infectious agents	Decontamination of Biological Safety Cabinet (BSC)	

References:

- http://generalbacteriology.weebly.com/sterilization-and-disinfection.html
- http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Projects00/sterilize/filtration.html
- http://textbookofbacteriology.net/control.html
- http://classes.midlandstech.edu/carterp/Courses/bio225/chap07/lecture4.htm
- https://www.ncbi.nlm.nih.gov/books/NBK214356/
- http://www.uvm.edu/~esf/emergencyred/biospill.html

~End of Worksheet~