Microbiology T

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solution to which all the necessary nu
nutrients, different categories of med

Categories

Complex media are rich in nutrients
enzymatically digested animal protei
to serve as the main carbon and ener
is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called complex.

Defined media are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines).

Selective/differential media are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives. The additives may be species- or organism-selective (e.g., a specific substrate, or an inhibitor such as cyclohexamide (artidione) which inhibits all eucaryotic growth and is typically used to prevent fungal growth in mixed cultures).

Media	Purpose
Complex	Grow most heterotrophic organisms
Defined	Grow specific heterotrophs and are often mandatory for chemoautotrophs, photoautotrophs and for microbiological assays
Selective	Suppress unwanted microbes, or encourage desired microbes
Differential	Distinguish colonies of specific microbes from others
Enrichment	Simlar to selective media but designed to increase the numbers of desired microorganisms to a detectable level without stimulating the rest of the bacterial population
Reducing	Growth of obligate anaerobes

The mixture of necessary nutrients can be used as a liquid medium, or a solidifying agent can be added. "Agar agar" is a natural polysaccharide produced by marine algae and is the most commonly used solidifying agent added to media (end concentration usually 1.5 % w/v). If hydrolysis of the agar is suspected, a silica gel is used as a replacement solidifying agent.

Protein Hydrolysates

Complex media contain often protein hydrolysate which are are excellent natural sources of amino acids, peptides and proteins in growth media. It is the most important source for nitrogenous nutrients. They are most often obtained by enzymatic digestion or acid hydrolysis of natural products, such as animal tissues, milk, plants or microbial cultures. The number of available protein hydrolsate, also called peptones, is enormous and can promote and sustain the growth of most common organisms. For the enzymatic digestion often papain, pepsin, trypsin or a mixture of enzymes of the pancreatic juice are taken. Below is a list of often used expressions and the definitions.

Term	Explanation
Trypic digested	Protein hydrolysate was produced by protein digestion with trypsin
Peptic digested	Protein was digested by pepsin
Pancreatic digested	Protein was digested by a mixture of enzymes of the pancreatic juice
Proteose Peptonet	A mixed enzymatically digestion of meat proteins. It is rich in peptides with the higher molecular weight.
Tryptone	Casein which was tryptic digested
Tryptose/Tryplose	A mixed enzymatically digestion of animal proteins. The digest conditions are such that it contains many different peptides including those of higher molecular weight (proteoses).

Aseptic Techniques

Before inoculation with the desired microorganisms, microbiological media and all materials coming into contact with it must be sterile. During any subsequent handling of the bacterial cultures, unwanted or contaminant organisms must be excluded employing aseptic techniques.

Sterilisation implies the complete destruction of all microorganisms including spores, this is accomplished by the use of heat, chemicals, radiation, filtration.

- Indicators
- Microbiology
- Phase transitions
- Precipitation
- Vitamins

Heat

Denatures and coagulates vital proteins. There are various forms of heat sterilisation.

Red Heat

Inoculating wires or loops are sterilised by holding them in a Bunsen flame until they are red hot.

Moist Heat

Bacteria are more readily destroyed by moist heat (steam) than dry heat. Usually used for the sterilisation of culture media, aqueous solutions and the destruction of discarded cultures. Air must first be removed in order to achieve the 121 °C necessary for successful sterilisation. This is accomplished by the use of an autoclave (the technical version of a pressure cooker), which follows automatic cycles of heating under pressure for the required time.

Dry Heat

Usually employed for materials which could either be corroded by steam or must remain dry before use. These include metal instruments, glass petri dishes, flasks and pipettes and cotton wool. In practice, dry heat sterilisation requires longer time intervals and higher temperatures than steam sterilisation, e.g.steam sterilisation 121°C for 15mins or dry heat sterilisation 160°C for 120 minutes.

Chemical

Usually employed for delicate equipment such as optical instruments and electrical devices which would be damaged by heat. Due to the toxicity of the chemicals used, this is not the most popular form of sterilisation. Chemicals employed include: gaseous ethylene oxide, which alkylates amino, sulfhydryl, carboxyl and hydroxyl groups of microbial cell compounds; formaldehyde, used as a fumigant; and hydrogen peroxide vapour used in aseptic packaging.

Radiation

Employed for heat-sensitive materials and for environmental samples such as soil and sediment where structural changes caused by heat need to be avoided. Two forms of radiation are used:

UV

Initiates the excitation of atoms which in nucleic acids leads to fatal mutations. UV light cannot penetrate materials so is used mainly for surface treatments e.g. laminar flow benches, and air and water.

Ionizing Radiation

Can penetrate samples, causing ionization within cells. Gamma radiation generated through a ^{60}Co a-source is used to sterilise complex matrices such as soil and foodstuff. Microorganisms show increased resistance to radiation under anoxic conditions (2-5x) and also in frozen samples.

Filtration

Filtration sterilisation operates through the exclusion rather than destruction of microorganisms. It is safe for the user and is employed for sensitive liquids and gases. Three types of filters are currently in use:

Depth Filters

These are made of columns packed with fibrous materials such as glass wool or cotton wool. The twisting and turning fibres entrap particles and so act as filters; they show little resistance to flow and are used mainly for gases or as pre-filters for membrane filters which are easily clogged.

Membrane Filters

Act by screening out particles. Their effectiveness depends on the size of the membrane pores and the electrostatic attractions present. The most commonly used filters in microbiology are usually made of cellulose acetate or cellulose nitrate.
Size of filter pores required to screen out:
Yeast 0.45 -1.2 µm
Bacteria 0.2 µm
Viruses and mycoplasmas 0.01-0.1µm
Membrane filtration is usually employed for heat-sensitive substances, e.g. vitamin solutions; the filters are heat-sterilised before use.

Nucleation Track (Nuclepore) Filters

These filters consist of very thin polycarbonate films which have been treated with nuclear radiation and then etched with a chemical to create very uniform vertical holes. They are employed for the same material as membrane filters but have the disadvantage that they are more easily clogged.

Media Supplements

Table of the most commonly used Media Supplements, Methods of Sterilisation, Solubilities

Substance	Solubility in Water at 25°C	Comments/Sterilisation
Actidione (Cyclohexamide)	2.1 g/100 ml	Destroyed by boiling in aqueous solution at pH 7 for 1 hr. Filter sterilise
L(+)-Arabinose	1 g/1 ml	
Arginine*	15 g/100 ml	Sat. solution bly alkaline, absorbs CO_2 pK_1, 2.18 pK_2 9.09 pK_3 13.2
Asparagine*	2.16 g/100 ml	Stable in aq sol. at 100°C, hydrolysed in acidic solutions
Biotin	22 mg/100 ml	pH of 0.01% sol. = 4.5 acidic solutions can be heat-sterilised
Cysteine*	soluble	Neutral slightly alkaline solution is oxidised to cystine pK_1, 1.71 pK_2 8.33 pK_3 10.78
Dextrin	soluble in 3 parts boiling water	
Ehrlichs reagent	soluble	
Fructose	soluble	
Fuchsin	1 g/7 ml	
Galactose		a) soluble in 0.5 parts water, freely soluble in hot water b) soluble in 1.7 parts water at 17°C

Glucose	1 g/1 ml	pH of 0.5 M aq solution = 5.9
Glycerol	miscible	
Glycogen	soluble	with opalescence
Lactose	21.6 g/100 ml	a) 1 g/5 ml b) 1 g/2.2 ml at 15°C
Maltose	soluble	mp 102-103°C
Mannitol	soluble	
Niacin	soluble	Stable to autoclaving at 120°C for 20 mins
Ornithine*	soluble	Aqueous solution alkaline pK, 1.94 pK_2 8.65 pK_3 10.76
Phenylalanine*		L 29.6 g/L at 25°C D 1 g/35.5ml at 16°C DL 14.11 g/L at 25°C pK_1 2.58 pK_2 9.24
Resazurine	insoluble in water soluble in dil alkali hydroxides	Indicator 0.1 g in 20ml 1 N NaOH + water up to 500 ml pH 3.8 = orange, pH 6.5 dark violet
Ribose	soluble	
Citric acid	soluble 59.2 % at 20°C	pH of 0.1 N solution = 2.2
Sorbitol	soluble Up to83%	mp 100/112°C
Starch	insoluble	
Sucrose	1g/0.5ml	
EDTA (disodium salt)	soluble	Used to complex iron in media
EDTA (acid)	insoluble	

All stock solutions of amino acids can be autoclaved at 120°C for 20 mins.

Interaction of Media Components

When undertaking research where medium composition plays an important role, e.g. toxicity studies, care must be taken to observe the various interactions which can take place between media components, for example;

Yeast, peptone and amino acids	Bind large amounts of divalent Hg, Pb, Ag and Cu ions
Glucose	exhibits non-selective metal binding which can cause metal deficiency of essential ions or decrease the toxicity of toxic ions.
EDTA	chelates essential trace metals causing deficiency; the effect can be reversed by the addition of other metals e.g. Cu and Fe.
NaCl	increases Zn toxicity (formation of a Zn-chloro complex), decreases Cd toxicity.
Agar	in solid medium, Sn toxicity increases (formation of a soluble toxic tinagar complex).
Silica gel	decreases toxicity of Sn, Cd, Pb, Ni and Zn divalent ions.
Phosphate	precipitation* of insoluble phosphates decreasing toxicity of toxic ions
	(e.g Cd and Pb) or causing deficiency of essential ions (e.g. Fe)
Carbonate	precipitation of insoluble carbonates decreased Pb toxicity
Citrate	non-selective metal binding
Tris	non-selective metal binding

* Can be avoided by addition of a HEPES buffer to reduce phosphate.